SAMPLE PREPARATION IN ENVIRONMENTAL ORGANIC ANALYSIS

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To Doreen

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Abstract

Sample preparation in organic analytical chemistry is often the most important stage in the entire analysis procedure. However, there has been little development in the techniques used in over one hundred years and solvent extraction is still the method of choice. Unfortunately, this is time-consuming and creates large quantities of organic solvents which are harmful to the environment. The work presented here investigates the use of several new instrumental sample preparation techniques including, supercritical fluid extraction, solid-phase extraction, solid-phase microextraction and microwave assisted extraction. All of the techniques significantly reduce organic solvent consumption whilst allowing greater sample throughput through faster extraction and a greater scope for automation. Supercritical fluid extraction, using carbon dioxide as a solvent, dominates the project and has been used to extract pesticides from water, both directly and in combination with solid-phase extraction. Direct extraction proved ineffective due to the extensive extraction time required, although the combined approach is shown to allow selective extraction of different classes of pesticides and herbicides to be performed by alteration of the supercritical fluid conditions. In addition, solid-phase microextraction technology, which requires no organic solvents, has been used to extract herbicides from the same matrix. After the optimization of operating conditions, the technique was capable of the automated extraction and analysis of the analytes at a concentration of 0.1 $\mu g\ l^{-1}$. Supercritical fluid extraction protocols have also been developed which are capable of extracting similar concentrations (if not greater) of polycyclic aromatic hydrocarbons from soils as compared to traditional Soxhlet extraction, with a significant reduction in the overall extraction time. The technique was also compared with a microwave assisted extraction method which allows the simultaneous extraction of multiple samples. The optimization of operating parameters in both techniques was facilitated by the use of experimental design procedures capable of interpreting data obtained from experiments involving the simultaneous alteration of operating variables. Each system was found to have their strengths and weaknesses which are discussed, however, in both instances the soil matrix was found to play an important role in extraction efficiency. Further studies involving the supercritical fluid extraction of pesticides from various characterized soils concluded that the soil matrix significantly affected the overall extraction performance which was found to be particularly dependent on soil organic matter.

Abbreviations

AE	Alcohol Ethoxylate
ANOVA	Analysis of Variance
APE	Alcohol Phenyl Ethoxylate
BET	Brunauer-Emmett-Teller
BPR	Back-Pressure Regulator
BTEX	Benzene, Toluene, Ethyl Benzene and Xylene
CCD	Central Composite Design
CEC	Cation Exchange Capacity
CI	Chemical Ionization
CP	Critical Point
2,4-D	2,4-Dichlorophenoxyacetic Acid
DCM	Dichloromethane
DCP	2,4-Dichlorophenol
DDD	1,1-Dichloro-2,2-Di(chlorophenyl)ethane
DDT	1,1,1-Trichloro-2,2-Di(chlorophenyl)ethane
DMP	2,4-Dimethylphenol
DNA	Deoxyribonucleic Acid
ECD	Electron Capture Detector
EEC	European Economic Community
EI	Electron Impact (Ionization)
ELISA	Enzyme-Linked Immunosorbent Assay
EPA	Environmental Protection Agency
EPC	Electronic Pressure Control
FID	Flame Ionization Detector
FTIR	Fourier Transform Infra Red Spectroscopy
GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
GPC	Gel Permeation Chromatography
GPR	General Purpose Reagent
HCH	Hexachlorocyclohexane
HMDS	Hexamethyldisilane
HPLC	High Performance Liquid Chromatography
Kd	Equilibrium Distribution Coefficient
K_{ow}	Octanol-Water Partition Coefficient
LC	Liquid Chromatography
LGC	Laboratory of the Government Chemist
LLP	Liquid-Liquid Partitioning

Log P	Log of K_{ow}
LSC	Liquid Scintillation Counting
MSD	Mass Selective Detector
NPD	Nitrogen-Phosphorus Detector
OCP	Organochlorine Pesticide
OPP	Organophosphorus Pesticide
PAH	Polycyclic Aromatic Hydrocarbon
P_c	Critical Pressure
PC	Personal Computer
PCB	Polychlorinated Biphenyl
PCDD	Polychlorinated Dibenzo-p-Dioxin
PCDF	Polychlorinated Dibenzofuran
PFA	Perfluoro Alkoxyethylene
PFTBA	Perfluorotributylamine
PTFE	Polytetrafluoroethylene
PVC	Polyvinylchloride
RNA	Ribonucleic Acid
RSD	Relative Standard Deviation
SCA	Standing Committee of Analysts
SDB	Styrene Divinylbenzene
SF	Supercritical Fluid
SFC	Supercritical Fluid Chromatography
SFE	Supercritical Fluid Extraction
SIM	Selected Ion Monitoring
SPE	Solid-Phase Extraction
SPI	Septum Programmable Injector
SPME	Solid-Phase Microextraction
SVOC	Semi-Volatile Organic Compound
2,4,5-T	2,4,5-Trichlorophenoxyacetic Acid
T_c	Critical Temperature
TIC	Total Ion Chromatogram
TMCS	Trimethylchlorosilane
TP	Triple Point
UV	Ultra-Violet
VOC	Volatile Organic Compound
XAD	Styrene Divinylbenzene Copolymer
XRF	X-Ray Fluorescence

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Disclaimer

The nature of the work contained in the thesis requires that many references are made to the pressure in a particular instrumental system. Unfortunately there are several different units in which pressure is routinely quoted in the literature and, more significantly in this case, different units in which pressure is read on analytical instruments. More specifically within the thesis, pressure is quoted in units of Pascals (Pa), atmospheres (atm), bar, pounds per square inch (psi) and kg cm^{-2}. The pressures quoted from original sources have been left in which ever unit they appeared as conversion to an universal unit may have caused calculation errors. In addition, the pressures used throughout the work have not been converted since the resultant values may have become difficult to understand and interpret. However, a table which allows all of the pressure units used to be converted to Pascals is included in appendix 7 (table A7.1), should the reader require pressures to be directly compared.

Contents

INTRODUCTION

Chapter 1

Organic Pollution in the Environment

1.1 Organic Priority Pollutants in the Environment

The world population's ever-expanding utilization of materials, energy, and space is accompanied by an increasing movement of man-made organic chemicals into the environment. These compounds encompass naturally occurring (mainly petroleum components) as well as synthetic chemicals. If the consumption of mineral oil is considered, (estimated to be in excess of 3 billion tons in 1992[1]) together with the exponential increase in the manufacture of synthetic chemicals over the past few decades, then it is obvious that such chemicals can lead to contamination of the soil, water, and air and will continue to be of environmental concern.

Of particular concern among the thousands of existing xenobiotic chemicals are the ones that are introduced into the environment in large quantities. The release of such chemicals is not solely due to large chemical industrialization processes, but is as a result of the use and uncontrolled release of compounds used in every-day life. Perhaps the most obvious example is the pollution of the environment with fossil fuels (liquid petroleum products and coal) and their combustion products. Such organic chemicals have been introduced into the environment in greater amounts by the increased use of motor vehicular transport and the growing demand for electricity, conventionally supplied by burning fossil fuels. One class of petroleum by-products that is of concern are the polycyclic aromatic hydrocarbons (PAHs) which are organic chemicals composed of fused benzene rings, and are produced by the incomplete combustion of organic compounds and are now ubiquitous in the urban atmosphere.[2] Emission of PAHs to the UK atmosphere from primary combustion sources has been estimated to be greater than 1000 tonnes total PAHs per annum.[3] Major sources of PAH pollution include vehicular traffic, use of fuel for industrial processes, residential heating (which occurs under less controlled, less efficient and lower temperature conditions than used in power stations, leading to PAH emissions that are significantly higher[4]), and waste incineration. However, PAHs have received much attention both because of their continual emission from combustion sources and also because of their biological activities.[5] Health concerns regarding PAHs focus on their metabolic transformation by aquatic and terrestrial organisms into toxic, mutagenic and carcinogenic agents such as dihydrodiol epoxides. These metabolites bind to and disrupt DNA (deoxyribonucleic acid) and RNA (ribonucleic acid) which is the basis for tumour formation. However, following ingestion by mammals and other life forms, PAHs are not bio-accumulated in the same manner as other organic pollutants, since they tend to be metabolized at the site of entry into the body. This class of pollutant ranges from the relatively low molecular weight two-ring system of

naphthalene to complex high molecular weight five- and six-membered ring systems. Therefore individual PAHs differ substantially in their physical and chemical properties which in turn dictate their environmental fate. The more volatile low molecular weight compounds dominate the overall atmospheric content, whereas the heavier PAHs are found mainly in soils and sediments and are strongly bound to soil organic matter. In fact, the total UK PAH burden in soils has recently been estimated to be approximately 50 000 tonnes,[3] with this estimate not taking actual contaminated land sites into consideration. Measurement of PAHs is therefore routinely carried-out in the air (as particulates) or from soil and dust samples, although they have also been reported to be present in common foods in the UK.[6]

Of equal concern to the environment are those organic compounds containing one or more halogens in their chemical structure (halogenated hydrocarbons). Polychlorinated biphenyl's or PCBs are a class of 209 discrete chemical compounds, called congeners, in which one to ten chlorine atoms are attached to biphenyl. PCBs were commercially produced as complex mixtures for a variety of uses with the major manufacturer producing PCBs under the trade name Aroclor from 1930 to 1977. Aroclors were marketed for use in transformers, capacitors, hydraulic fluids, lubricants, and as additives in printing inks, paints, plastics, and adhesives as well as other applications. In addition to their manufacture, PCBs may also be produced as unwanted by-products from a variety of chemical processes. Such sources include PCB formation from the thermal degradation of other chlorinated organic compounds in waste incineration and the production of the pollutants during water chlorination.[7]

The chemical and physical stability of PCBs together with their electrical insulating properties led to their commercial utility. However, these properties have also been responsible for PCB environmental contamination problems. Since PCBs do not readily degrade in the environment after disposal and are lipophilic, they are persistent and tend to bioaccumulate. PCBs were found in fish, birds, and other environmental samples as early as 1966.[7] More recently, PCBs have been shown to be almost ubiquitous environmental pollutants, occurring in most human and animal tissue, milk, sediment and many other matrices. Public and scientific concern about PCBs arose from the findings that PCBs were toxic and therefore undesirable as commercial products or environmental contaminants. Studies on the individual PCB congeners indicated that toxicity depended not only on the degree of chlorination but also on the isomer. Effects of PCB contamination in animals has ranged from reproduction and growth abnormalities (monkey, dog, and wildfowl) to liver damage (rat) and mutagenicity. Workers in production plants routinely exposed to high levels of PCBs

showed external symptoms including burning of the eyes, face and skin, together with clinical symptoms of PCB-related liver injury. Although there has been some evidence of an increased incidence of cancer the findings have been inconclusive.[8]

Other halogenated hydrocarbons that are of great environmental concern, particularly regarding human health, include the class of compounds known as dioxins. Dioxin is a synonym for polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). Of the 75 possible PCDDs and 135 possible PCDFs, environmental interest has centred on those with four or more chlorine atoms.[9] The sources of the dioxins in particular result from the use of organochlorine chemicals and include waste incinerators (municipal, chemical, and clinical, ranked in descending order of total emission) coal combustion, and the use of chlorinated compounds in the chemical industry. Dioxins are mainly found in soil, generally in urban areas, although they have been detected in water, air, and of greater concern, human milk. The main reason why dioxins have evoked such interest is their acute toxicity to laboratory animals. In fact, dioxins were once widely thought of as the most toxic chemical known to man and were a contaminant of the infamous Agent Orange defoliant (the herbicide 2,4,5-T) used in the Vietnam war. However, the toxicological evidence based upon experimentation involving guinea pigs may not extrapolate well to humans and it has been reported that the US Environmental Protection Agency (EPA) has postulated that some of the dioxin congeners may not be as toxic as once feared.[10]

The second category of organic chemicals that are of major concern to the environment are those which are synthetically manufactured and not produced as by-products in industrial processes. This wide classification of organic pollutants encompasses a great number of compounds which fall into many different groups. However, several classes of compounds are of particular environmental concern.

Halogenated short chain aliphatic hydrocarbons are one group of chemicals that is used extensively. These compounds are generally inert, non-flammable, and, depending on the type and number of halogen substituents, exhibit physical properties that render them unique for use as either aerosol propellants, refrigerants, blowing agents for plastic foams or solvents for many applications. Their chemical inertness and the large quantities used has meant that they have become major environmental pollutants. Examples within the group include fluorocarbons (freons) which have been shown to deplete the stratospheric ozone layer, and chlorinated solvents (*i.e.*

dichloromethane and tetrachloroethane) which are among the top ten organic groundwater pollutants.[11]

Another group of high-volume chemicals which are ubiquitous in the environment are the phthalates, which are diesters of phthalic acid. The annual production of phthalates exceeds 1 million tonnes and they are mainly used as plastisizers, in particular, to make polyvinylchloride (PVC) flexible.[11] In addition, they are among the most commonly found chemicals in the analytical laboratory since they are obtained from plastic bottle tops and seals often used in sample storage.

Most of the chemical pollutants already discussed have been relatively non-polar in nature and therefore hydrophobic. There are, however, many chemicals which exhibit hydrophilic structures which are of environmental concern. One important example are the surfactants, which consist of non-polar and polar sections and are usually produced by combining a large hydrocarbon chain with a polar group that can be either anionic (negatively charged), cationic (positively charged) or non-ionic (neutral). Owing to their amphiphilic nature, surfactants have unique properties and in aqueous solutions they distribute in such a way that their concentration at the interface between a gas or solid is higher than in the bulk of the solution. Also, upon reaching certain concentrations in the bulk phase, surfactants form aggregates (micelles) that may keep otherwise insoluble compounds in the aqueous phase. Therefore, they are an important part of any detergent and are also widely used as wetting agents, dispersing agents, and emulsifiers. Unfortunately, because of their direct use with water, surfactants are discharged into municipal and industrial wastewater's and often constitute a major proportion of the total carbon content found in polluted water.[12]

Within the group of synthetic organic pollutants, pesticides, which are by their nature of particular importance to humans since they are commonly applied to food crops, require special consideration. Pesticides are a series of compounds considered collectively because of their use. Chemically they are a diverse series of chemicals with varying properties and contain different classes of compounds including insecticides, fungicides, and herbicides. Different forms of crop protection chemicals have been used throughout civilization with their beginning probably occurring in ancient Greece where sulphur was used as an insecticide.[13] Inorganic pesticides were continually used to protect crops, including some which were extremely toxic to humans (arsenic and cyanide) which caused harmful residues to be present in the actual crop. It was not until the 1930's that examples of the modern era of synthetic organic pesticides were first developed. However, when viewed from the present day,

developments in pesticides were slow, and by 1939, only around thirty were registered for use in the United States. Application techniques were limited to small sprayers and dusters, and applying pesticides was time consuming and the area of crops treated relatively small.[14] Under these circumstances the environmental impact of pesticides was small and restricted to the immediate area of application. The advent of World War II placed a strain on food resources with tight control on shipping restricting the availability of imported pesticides, and in Europe and North America pest control became more difficult. In Germany research to find a replacement for pesticides no longer available led to the development of dichlorodiphenyltrichloroethane or DDT, by Dr Paul Muller in 1939. Following successful trials against the potato beetle, DDT production began in 1943 and soon became the most widely used single insecticide in the world.[13] DDT was the first of the "new" insecticides, and because of the time was not only used to produce pest free crops but also in the war effort where it was used to fight human diseases carried by insects (including typhus and malaria). In fact, it has been postulated that DDT has saved more lives than any other chemical.[15] However, the use of DDT was not without its problems. After only a few years of using DDT, certain insect strains became resistant to the compound. In addition, DDT is a rather broad spectrum insecticide which is naturally persistent in nature and was found to affect non-target species. DDT which had been used on animal feed was soon found in the tissues of the animals consuming the feeds and in products such as milk and eggs that they produced. Relatively low concentrations of the compound were multiplied by bio-concentration within the fat tissue of the animal ingesting the contaminated food, leading to unusually high amounts in milk used to feed the very young.

Following the initial success of DDT, other organochlorine pesticides (OCPs) were developed around the same time. Hexachlorocyclohexane (HCH) was first used for its insecticidal properties in 1943 (although it had been prepared in 1825 by the English chemist Michael Faraday). The ring that forms the framework of HCH is not planar but chair-shaped and eight forms of HCH can therefore exist with the chlorine and hydrogen atoms in various axial and equatorial positions. Of these isomers, only the γ isomer (more commonly known as lindane) is highly toxic to insects, being for many insects from 50 to several thousand times more toxic than the α or δ isomers, although the exact reason for this increased toxicity is unknown.[16]

From about 1945 several insecticidal chlorinated hydrocarbon cyclodiene compounds were introduced, although they did not come into widespread use until the middle 1950's. Common examples include, aldrin, dieldrin, heptachlor, and endrin. As with HCH they are not planar but have two such rings that are fused together. Both rings

are "bridged" and the fusion can occur in more than one way with respect to the bridge and the curvature of one ring to the other. Aldrin and dieldrin are stereochemically related with the latter having an epoxide ring in the second non-planar ring. Similarly, isodrin is epoxidized to endrin and in each case the former is less polar and has a higher vapour pressure than the epoxide compound. This difference accounts for the use of aldrin as a soil fumigant to control wireworms and the larvae of root flies whereas dieldrin has been more often employed as a root dip and as a seed dressing. Dieldrin has also been successfully used to control external parasites on farm animals and is used in sheep and cattle dips.

Despite the early promise of the organochlorine insecticides, their use is currently under review due to their pollution of the environment. In soil, most organochlorine compounds persist for months or even years. This is especially so when high doses are applied to clay soils or to soils rich in organic matter. In addition, DDT is not the only organochlorine insecticide that is widely effective against non-target organisms. Both lindane and the chlorodiene family of compounds have been detected in many wildlife tissues, and have been associated with numerous fish kills. Also, several types of birds (including the peregrine falcon and sparrowhawk) were noted to be in decline, and relatively early studies in England, pointed to the pesticides as the responsible agents.[17]

The early discovery of insect immunity towards certain organochlorine insecticides (namely DDT) fuelled the search for different types of insecticide which were as effective as DDT but less persistent in the environment. The organophosphorus compounds represent another extremely important class of organic insecticides and were first developed from wartime research into chemical nerve agents. However, these first generation organophosphorus pesticides (OPPs) showed a high toxicity towards mammals and later research in this field was directed towards the discovery of more selective, less poisonous insecticides. Malathion (discovered in 1950) was the first example of a wide-spread organophosphorus insecticide combined with a very low mammalian toxicity. An important advantage of the organophosphorus insecticides is that after application they are generally rapidly degraded to non-toxic materials and consequently are not persistent (unlike organochlorine insecticides) and therefore do not tend to accumulate in the environment and along the food chain. An important feature of this group of insecticides is that different members possess very different physiochemical properties, in particular, they have different vapour pressures at room temperature and solubilities in water. The basic structure of an organophosphorus insecticide is shown in figure 1.1.

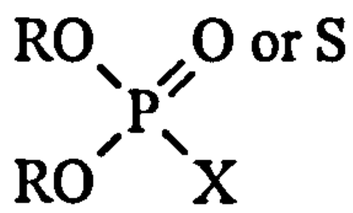


Figure 1.1 General Structure of an Organophosphorus Insecticide.
(where; the two R groups are usually methyl or ethyl and the same in any one molecule, and X is frequently a complex aliphatic or aromatic group)

This wide spectrum of physiochemical properties enables appropriate substances to possess a correspondingly wide range of uses in both agriculture and animal hygiene. Some are used as fumigants (dichlorvos, used for its high vapour pressure), others as contact poisons (*i.e.* mevinphos, used because of its low chemical stability), and others as persistent contact insecticides (*i.e.* malathion and diazinon, used because of there high chemical stability and low water solubility which enables them to soak into the leaves of the plant but not to travel around it). Consequently, organophosphorus compounds have overtaken organochlorine compounds as the most-used insecticides, even though they are generally more expensive and may require an increased application rate.

The rapid developments in insecticides have been paralleled by new discoveries of compounds effective for plant disease and weed control. In the latter case the search was fuelled by rising labour costs that made hand-weeding too expensive, and the early discovery of 2,4-dichlorophenoxyacetic acid or 2,4-D (1943) and triazines provided selective herbicides that greatly contributed to the mechanization of crop production and the efficiency of labour usage. The first triazine herbicide, chlorazine was introduced in 1954, and this was followed by simazine in 1955.[18] Whereas 2,4-D and other phenoxyacetic acid derivatives are effective against broad-leaf weeds, the soil applied triazine herbicides are effective in many different situations. As a result of their broad application, herbicides are the most extensively used pesticides in agriculture with their use in North America exceeding that of insecticides and fungicides combined.

Most herbicides based on the symmetrical triazine nucleus have alkyl-substituted amino groups in the 4 and 6 positions and either a chlorine group or a methylthio group in position 2. This is illustrated in figure 1.2. The compounds containing a chlorine atom have names terminating in -azine and those containing a methylthio group end in -etryn(e).

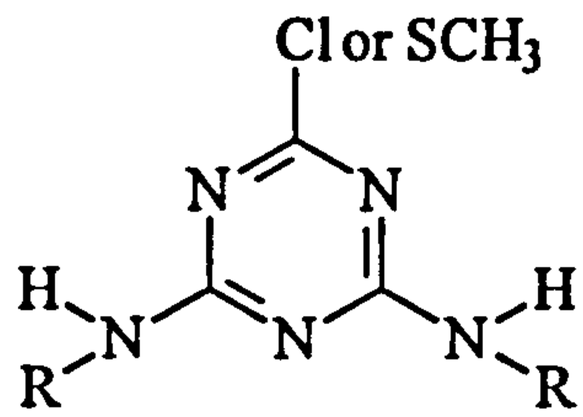
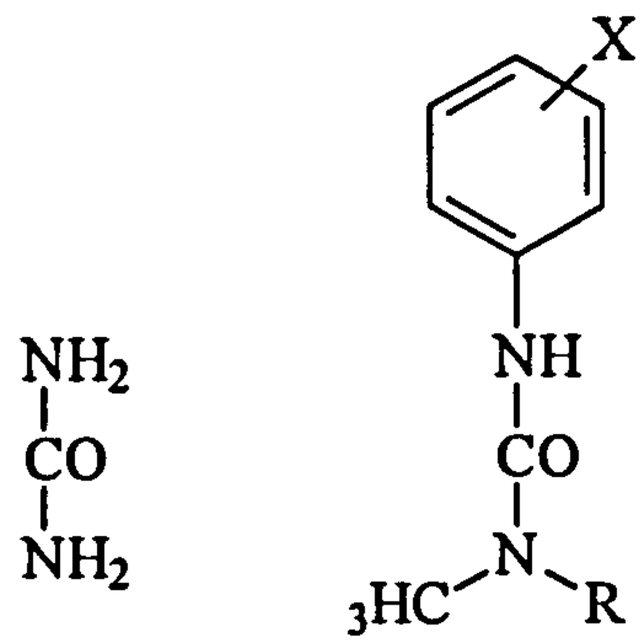


Figure 1.2 General Structure of a Triazine Herbicide.
(where; R is an alkyl group commonly, methyl, ethyl, or isopropyl)

Some crops are tolerant to specific triazines. However for most crops, the apparent selectivity of the herbicide in the field depends largely on the crop being large-seeded, or consisting of bulbs and consequently more deeply placed in the soil than seedling weeds. Triazines have a wide spectrum of activity, killing many annual grasses as well as ragweed and nightshade, with specific examples including the use of simazine for total weed control in non-crop situations and atrazine to kill weed seedlings in maize and sugar cane. However, they can persist for many months in some soils and cause seasonal carry-over problems.

One other common class of herbicide is the urea herbicide, so-called because urea is tri-substituted as indicated in figure 1.3. One of the amino groups carries either two methyl or one methyl and one methoxy group. The other amino group is substituted with a benzene ring which, in most cases, contains halogen atoms.



A **B**

Figure 1.3 General Structure of an Urea Herbicide.
(where; **A** is Urea, and **B** is the urea herbicide containing R = CH_3 or OCH_3, and X = Cl, Br, $CH(CH_3)_2$, or CF_3)

As with the triazine herbicides, the urea herbicides are selective in their use although selectivity is reduced as the application rate is increased. Some control, with equal effectiveness, seedling grasses and a range of broad-leaved weeds (diuron) whereas others are more selective in their action (fluometuron). Urea herbicides can persist in the soil environment with the level of persistence being decided by the organic content of the soil and the amount of rain falling in the first few weeks after application, as well as the actual herbicide applied. Both the urea herbicides, and the triazine herbicides kill weeds by disrupting photosynthesis, which is the process by which green plants use light energy to convert carbon dioxide and water into carbohydrates.

Despite the many years of research that is devoted to the physiochemical and biological effects that a new pesticide has on the environment, it has been shown that there are many problems associated with the wide-spread use of pesticides throughout the world. Either by their misuse or because they are persistent in the environment, pesticides find themselves in the crops and animals which we eat and in the watercourses where drinking water is ultimately obtained. Pesticides that are applied to soils, plants, or water can affect animals and their delicate interrelationship either indirectly or directly. The main problem with the use of pesticides, and with the other pollutants discussed, is that they are capable of moving away from the area of their initial source. If the mechanisms by which the movement has occurred are unknown, or if the class of pollutant is persistent in the environment, the pollutant can become a major cause for environmental concern.

1.2 The Loss and Movement of Pollutants in the Environment

The physical and chemical properties of most organic pollutants, particularly hydrophobic, semi-volatile compounds (*i.e.* PCBs and PAHs) and many classes of pesticide (organochlorine pesticides, and most organophosphorus pesticides or herbicides) ensure they find their way into the soil environment, regardless of their initial source or site of application. Therefore it is their movement and release from soil which is of interest when assessing the fate of organic chemicals in the environment. Organic chemicals can be attached to the soil, be removed from their initial position, or be transformed by a variety of physical and chemical processes, which may lead to the subsequent contamination of watercourses or the atmosphere. The characteristics of a soil therefore often dictate the eventual fate of an organic chemical, and the main constituents in soil are discussed.

Clay The term "clay" is usually used to define crystalline minerals, and crystalline amorphous oxides and hydroxides. Clays represent layers of silica and aluminium sheets where the silica consists of a silica atom surrounded by four oxygen atoms in tetrahedral symmetry, and alumina represents aluminium atoms co-ordinated by six oxygen or hydroxyl atoms in octahedral symmetry. In the clay material the silica and alumina sheets are usually in a 1:1 or 2:1 ratio. An example of a 1:1 clay is kaolinite and is made up of one sheet of tetrahedrally co-ordinated cations (Si^{4+}) with one sheet of octahedrally co-ordinated cations (Al^{3+}). The surface on the layer of the alumina side is composed of hydroxyls and on the silica side of oxygen. The 2:1 clay minerals, such as montmorillonite are made up by combination of two tetrahedrally co-ordinated sheets of cations, one either side of an octahedrally co-ordinated sheet. In many cases the aluminium and silica atoms are replaced by such atoms as iron and magnesium which produce a change in the net charge on the clay surface.[19]

Almost all soils contain at least a small proportion of colloidal oxides and hydroxides. The crystalline and amorphous oxides and hydroxides of aluminium, iron, and silica occur in soils as separate phases as well as coatings on surfaces of other phases. Soils containing high amounts of oxides and hydroxides may differ in their adsorptive properties from mineral and organic soils.[19]

Organic Matter Soil organic matter plays an important role in affecting the fate of organic pollutants in the soil environment. Organic matter in soils is commonly known as humic substances which are formed by the degradation of plant and animal tissues. The major constituents of humic substances are acidic, dark coloured, predominately aromatic, chemically complex, hydrophobic materials that range in molecular weight from a few hundred to several thousand atomic mass units. The minor constituents of humic substances are those with definite chemical characteristics such as carbohydrates, proteins, amino acids, fats, waxes, and low molecular weight organic acids.[19]

The complex nature of soil organic matter has led to characterization by its solubility in acid or alkali, into three main fractions. Humic acid is soluble in dilute alkali but is precipitated on acidification of the alkaline extract. Fulvic acid is that humic fraction remaining in solution when the alkaline extract is acidified (soluble in both dilute acid and alkali). Finally, humin is that humic fraction that cannot be extracted from soil by dilute acid or base. Structurally the three humic fractions are similar, but differ in molecular weight and functional group content, with fulvic acid having a lower molecular weight but higher content of oxygen containing functional groups per unit

weight. The chemical structure and properties of the humin fraction are similar to humic acid, with its insolubility arising from it being adsorbed or bonded to inorganic soil constituents. A typical structure of humic acid proposed by Kononova is given in figure 1.4.[20] Humic substances usually possess large surface areas (500 - 800 m^2g^{-1}) and cation exchange capacities.

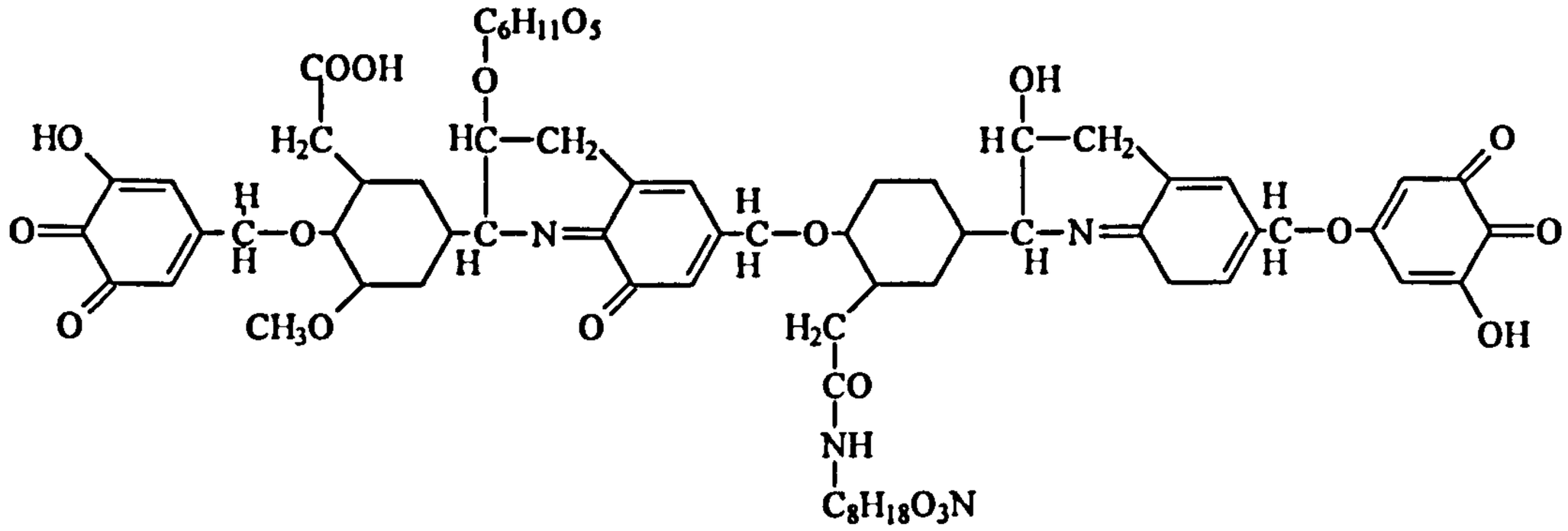


Figure 1.4 Proposed Structure of a Humic Acid.

The mechanisms by which organic chemicals are associated with soil together with how the loss and movement of organic pollutants occur from the soil environment are discussed below.

1.2.1 Mechanisms of Pollutant Sorption in Soils

The process in which chemicals become associated with solid phases is generally referred to as sorption, which may be either adsorption (on a two-dimensional surface) or absorption (into a three-dimensional matrix). Sorption is extremely important because it may dramatically affect the fate of chemicals in the environment since chemically identical molecules behave very differently if they are bound to the exterior of a solid (or absorbed within the soil matrix) than when they are in solution. Obviously the movement of water-borne molecules differs from those same molecules carried by particles that settle in sediment. In addition, only dissolved molecules are available to cross phase boundaries and therefore volatilization cannot take place if chemicals are sorbed to solids. Similarly transformation of a chemical, either by photodecomposition or biological or chemical degradation, may be severely reduced for sorbed species. However, the chemical environment surrounding a sorbed molecule may be different to that of the bulk solution and can actually promote chemical reaction.[21]

Sorption is not always a single simple process, but some combination of interactions may be responsible for dictating the overall association of a chemical to a solid. The way in which a compound is sorbed to a solid is not only dependent on the physical and chemical properties of the chemical but also on the composition of the solid phase. In soil, the solid phase mainly consists of clay minerals, organic matter and oxides / hydroxides of aluminium and silicon, which make up around 50 % of the soil volume (the other half being filled by the soil solution and air).[19] The two main components in soil which are responsible for sorption are organic matter and clay.

The weak intermolecular interactions responsible for sorption in soils are discussed below. Most of the interactions discussed are relatively weak when compared to ion-ion interactions which have a typical energy of approximately 250 kJ mol^{-1}.[22]

Van der Waals Forces[22]

All compounds experience van der Waals forces or dispersive attractions towards one another. Even non-polar substances, which exhibit a time-average smooth distribution of electrons throughout their structure, have instantaneous displacements of their electrons such that momentary electron-rich and electron-deficient areas develop giving a transient dipole. This momentary distribution of charges is felt by neighbouring molecules whose electrons respond in a complementary way. Consequently, there is an instantaneous intermolecular attraction between these regions, with the attraction shifting elsewhere in the molecule within the next moment. Typical energy values for van der Waals interactions are around 2 kJ mol^{-1} between all types of molecules and are therefore relatively weak (although summed).

Dipole-Dipole Interactions[22]

Owing to the differing electron-attracting properties (electronegativities) of the various types of atoms included in organic compounds, structures with different atoms bound to one another exhibit regions which are either deficient or always rich in electrons. Therefore in addition to the van der Waals forces always present, the dipole aligns itself with other dipoles in a head-to-tail arrangement resulting in dipole-dipole interactions. Energies of dipole-dipole interactions range from 2 kJ mol^{-1} between stationary polar molecules and 0.3 kJ mol^{-1} between rotating polar molecules.

Dipole-Induced Dipole Interactions

If a dipole is positioned near an evenly charged structural region of an adjacent molecule, electrons in the neighbouring molecule will be displaced in response to the approaching polar region. A polar molecule with a dipole moment can therefore

induce a dipole in a polarizable molecule and the two are attracted together with an average energy of around 0.8 kJ mol^{-1}, depending upon the two molecules.

Hydrogen Bonding[22]

The interactions described above are universal in the sense that they are possessed by all molecules and independent of their identity. However, a hydrogen bond can obviously only be possessed by molecules containing hydrogen atoms and is an attractive interaction that arises from a link of the form A-H.....B, where A and B are highly electronegative elements and B has a lone pair of electrons. Hydrogen bonding is conventionally regarded as being limited to nitrogen, oxygen, and fluorine but, if B is an anionic species (such as Cl^-), then hydrogen bonding may occur. The substantial differences in electronegativity between hydrogen and oxygen, or nitrogen mean that hydrogens bound to oxygen in alcohols or nitrogen in amines are particularly electron deficient and "protrude" from the basic structure. Neighbouring molecules with substituents containing oxygen or nitrogen and their non-bonding electrons, can engage these available hydrogens and form a hydrogen bond. The strength of the hydrogen bond is found to be about 20 kJ mol^{-1} which is far greater than the energy of the other interactions described and if hydrogen bonding is present it dominates van der Waals interactions.

Charge Transfer

In the formation of charge transfer complexes, electrostatic attraction takes place when electrons are transferred from an electron rich donor to an electron deficient acceptor. Charge transfer interaction only takes place within short distances of separation between interacting species. The formation of charge transfer complexes has been postulated as a possible mechanism involved in the adsorption of s-triazines onto soil organic matter and clay minerals.[23] The charge transfer reactions are particularly important when explaining the high adsorption of the methylthiotriazines onto organic matter. In addition, substituted urea herbicides also possess electron donor capacities and may interact with electron-acceptor moieties in humic acid to form charge transfer complexes.[24]

Ion Exchange

Ion exchange sorption can take place for those pollutants that can either exist as anions or that become positively charged through protonation. The adsorption is always accompanied by the release of a significant concentration of hydrogen ions. Examples include the adsorption of cationic pesticides such as diquat (1,1'-ethylene-2,2'-bipyridyldiylium ion, usually used as a dibromide compound[25]) *via* cation

exchange functions through -COOH and phenolic -OH groups associated with organic matter. Ion exchange has also been responsible for adsorption of less basic pesticides, such as s-triazine herbicides on organic matter and clay minerals. The pesticides may become cationic through protonation either in the soil solution or during adsorption with the process depending upon the pH of the system as well as the pKa of the specific compound. Evidence that the maximum adsorption of s-triazines on organic soils occurs at pH levels close to the pKa of the herbicide is indicative of ion-exchange mechanisms. However, the pH of the humic substance may be significantly lower than that of the liquid phase, thus surface-protonation of a basic molecule may occur even though the measured pH of the medium is greater than the pKa of the sorbate.[24]

Ligand Exchange

Adsorption by ligand exchange mechanism involves the replacement of hydration of water or other weak ligands partially holding polyvalent cations associated with the soil organic matter by suitable adsorbent molecules such as s-triazines.[19]

Hydrophobic Sorption and Partitioning

The interaction of organic pollutants with soil organic matter is usually governed by the aqueous solubility of the pollutant. Neutral, relatively non-polar compounds such as organochlorine and organophosphorus pesticides, or PAHs do not readily dissolve in water. This incompatibility principally arises because water molecules change their overall hydrogen-bonding to their surroundings when they are forced to interface with such non-polar solutes or non-polar structural sub-groups, and the solutes are said to be "hydrophobic". In a similar manner, most natural minerals are polar and expose a combination of hydroxy and oxy groups to their exterior. These polar surfaces strongly favour interactions which allow them to form hydrogen-bonds and liquid water is strongly adsorbed. Therefore, replacing water molecules at a mineral surface with non-polar organic compounds is energetically unfavourable, despite the hydrophobic nature of the chemicals. However, sorption of neutral organic chemicals into any natural organic matter included in the solid phase does not require displacement of tightly bound water molecules. The predominately aromatic structure of humic organic matter (comprising 40 - 50 % carbon by weight) is only able to hydrogen-bond at limited points, and offers a relatively non-polar environment in which hydrophobic compounds may be sorbed without undue competition with water. It is not surprising therefore that neutral chemicals show a greater sorption to soils and sediments that contain high amounts of natural organic matter.[11,26]

In some instances, soils do not include important amounts of natural organic matter. Consequently, sorption of organic solutes, especially hydrophobic ones, with mineral surfaces becomes significant. Most minerals expose a surface to the exterior which consists of hydroxyls protruding into the medium from a plane of electron-deficient atoms (Si, Al, Fe) and electron-rich ligands (oxygen). Like water molecules, these surface hydroxyls prefer to form hydrogen bonds with the molecules adjacent to the mineral surface. While all sorbates are attracted to the surface by van der Waals forces, as functional groups capable of dipole - dipole interactions and hydrogen bonding are added, stronger attractions per unit surface area are observed. Therefore interactions may vary from simple van der Waals dispersive forces between a mineral surface and hexane, to dipole - induced dipole interactions between the permanently charged surface ($Si^{\delta+}$-$O^{\delta-}$) and a polarizable aromatic ring of a pollutant. The situation may be further complicated if organic sorbates possess hydrogen atoms or lone pairs of electrons which are capable of forming hydrogen bonds with the mineral surface. Examples of such compounds include organic chemicals which possess alcoholic groups or amino functional groups such as triazine herbicides.[26]

1.2.2 Movement of Pollutants in Soil

The movement of an organic pollutant in the soil environment may occur while in solution or adsorbed on migrating particulate matter, by volatilization, or the chemical may be converted by a transformation reaction.

Diffusion and Mass Flow

Movement through soil in the solution phase may involve diffusion and mass flow processes. Diffusion is the process by which molecules are transported as a result of random molecular motion caused by their thermal energy. Thus, there is a net movement from positions of high concentrations to positions of low concentrations. A number of soil and environmental factors influence the diffusion of pollutants in soil. These factors are diffusion coefficient, solubility, sorption, bulk density and soil water content and porosity. Mass flow occurs as a result of external forces acting on the carrier for the pollutant in question. The pollutant may be associated with water as a solution, suspension, or emulsion and in porous soil environments, mass flow is the dominant transport mechanism for the movement of dissolved pollutants.[27] Solutes are transported by the bulk flow of groundwater flowing from regions of the sub-surface where water levels are high to the regions where water levels are low. Groundwater velocities depend upon the gradient in which they flow and on the

porosity of the medium through which they are flowing. Typically, groundwater velocities through uniform sand and gravel soils are between 10 and 100 m $year^{-1}$, with a potential range of between 1 and 1000 m $year^{-1}$. However, dense organic liquids may migrate downwards with a dominant vertical component, even if groundwater flow is horizontal. Surface run-off may also be a major route for pollutant movement and is dependent upon the amount of precipitation within the contaminated area.

Sorption is the most important factor influencing the mass transport of a pollutant through soil by water, with an inverse relationship between the amount of sorption and the rate of movement through the soil environment.[19] However, the soil particles onto which the chemical is sorbed, may act as a carrier when moved by water. Pollutants which are most likely to be moved by this mechanism are those which are not mobile.

Volatilization

Volatilization is the process whereby a compound evaporates in the vapour phase to the atmosphere from another component of the environment. Volatilization is an extremely important form of diffusion for many organic chemicals, although the process is less significant for inorganic compounds. Three sets of factors affect the volatilization process.[27]

1. Those which affect movement away from the evaporating surface into the atmosphere. Atmospheric conditions determining air flow over the soil surface play an important role in the removal of vapour from the soil surface.

2. Those which affect the vapour density of the chemical. Temperature primarily affects vapour density, with an increase in temperature normally increasing the equilibrium vapour density.

3. Those which control the rate of movement to the evaporating surface. Sorption of the chemical to the soil or water phase dictates the rate in which the compound is capable of reaching the evaporating surface where volatilization occurs. Therefore an inverse relationship between the soil organic content and volatilization is often noted.[19] The water content of the soil may also affect volatilization losses by competing with soil sorption sites. In addition, when water evaporates from the soil surface, an upward movement of water results in order to replace that evaporated

water. Thus, chemicals in the soil solution will move towards the surface by mass flow of evaporating water.

Although volatilization is obviously an important process for volatile organic compounds with high vapour pressures, it may still occur with semi-volatile compounds in aqueous environments because of their low water solubility.

Pollutant Conversion and Degradation

The conversion of xenobiotic pollutants in the environment can occur by three main routes; chemically, photolytically, or biologically. Chemical conversion and degradation of compounds in soil is widespread and plays an important role in the dissipation of many chemicals in soil. Most of the reactions occur in water as the reaction medium, as the reactant, or as both. Hydrolysis is one of the most common conversion pathways of organic chemicals and is responsible for the degradation of some organophosphorus insecticides (diazinon and malathion).[19] The chemical hydrolysis of s-triazine herbicides also plays a major role in their chemical degradation and is dependent on the soil organic matter content (the organic matter catalyses the reaction) and on the soil pH. Other examples of hydrolysis include the rapid replacement of chloro groups in lindane by hydroxy groups in moist soils. Oxidation and reduction are also common chemical reactions in the soil environment and account for the degradation of DDT to some of its metabolites (reductive dechlorination of DDT to DDD and oxidation of DDT to dicofol).[16]

Photodecomposition is responsible for many chemical changes of pollutants in the environment. Within the range of ultraviolet sunlight wavelengths (290 to 450 nm), sufficient energy exists to bring about chemical transformations with the degradation products often being identical to those produced by chemical and biological reactions. For photodecomposition to occur, light must come in contact with the chemical and therefore in the soil environment, photodecomposition of pesticides is limited to those on or very near to the surface. The extent of photodecomposition depends on the duration of exposure, the intensity and wavelength of the light, the nature of the supporting medium or solvent, pH of the solution and the presence of water and air. Examples of photodecomposition reactions include the photolysis of endrin (an organochlorine insecticide) to form a ketoendrin and the photolysis of atrazine in water to yield the 2-hydroxy analogue only. However, photolysis under the same conditions in the presence of fulvic acid has been shown to N-dealkylate triazine herbicides.[19]

Another set of transformations that remove organic compounds from the environment is that group of reactions mediated by organisms. As with chemical and photolytic reactions these biological transformations change the structure of the pollutant but do not necessarily mineralize it (the process by which an organic chemical is completely degraded to stable inorganic forms). Biological transformations are particularly important because many reactions, although feasible, occur at extremely slow rates. Organisms enable such reactions to proceed by two different approaches. The first is by the use of special proteins called enzymes that serve as catalysts by facilitating interactions and therefore lowering the required activation energy. Secondly, organisms may use energy to convert chemicals that may interact with pollutants to form more reactive species. Factors which affect the biological degradation of chemicals include pH, temperature, extent of adsorption, moisture, and soil type.[28] Most organic chemicals undergo some form of biological degradation in the environment. Specific examples include the s-triazine herbicides where N-dealkylation is the major degradation pathway. Phenylureas are another group of herbicides which are biologically transformed by a large number of fungi and bacteria which are capable of demethylating diuron and linuron. Insecticides are also degraded in soil by micro-organisms. Loss of lindane in soil is attributed to slow bacterial decomposition by either successive dechlorination or conversion to other isomers of hexachlorocyclohexane, with the degradation of lindane occurring more rapidly in submerged rather than aerated soil. Cyclodiene organochlorine insecticides (including aldrin and dieldrin) are also readily biologically metabolized in soil with aldrin being converted to dieldrin by oxidation mediated by soil micro-organisms. Further break-down of dieldrin is however very slow with the chlorinated ring being stable, although a small amount of dieldrin (1 to 6 %) has been reported to be biologically converted to its diol.[29]

1.3 Modelling the Effects of Environmental Pollution

Ever since the discovery of trace residues of pesticides and other organic pollutants in water courses there has been increased concern in the fate of organic chemicals in the environment. Much research interest has been aimed at understanding the processes that control the penetration of pollutants into soil and the subsequent contamination of ground and surface water. The majority of the work has taken place in the laboratory or in relatively small field trials and has been used in order to develop some predictive capability to assess the potential risk pollution may pose to humans and to the environment.

1.3.1 Soil Adsorption Studies

Laboratory studies provide a basis for understanding the fate of organic chemicals in the environment. For a given chemical, the experimental determination of the equilibrium distribution coefficient, *Kd* between the soil and the aqueous phase gives an estimation of the affinity the chemical has for the soil environment. *Kd* is calculated using equation 1.1.

Kd = concentration in the solid phase / concentration in the aqueous phase (1.1)

The characteristic value of *Kd* for a particular pollutant may therefore be of use in determining the rate of movement of a pollutant away from its initial source. Unfortunately, as well as knowledge of the *Kd* value of a pollutant, a true assessment of the likelihood of water contamination in a particular situation requires other detailed information. This includes information concerning the soil and an understanding of the hydrogeological characteristics of the site. Only when all of this information is available, can a true assessment of risk be attempted. The value of *Kd* may also be of use when assessing the ease in which chemicals are subsequently removed from soil, for example in an extraction process. Those chemicals with high *Kd* values (great affinity for the soil phase) will be more difficult to remove during an extraction than those with smaller *Kd* values. Equilibrium distribution coefficients are commonly derived using batch equilibration procedures where a known amount of soil and water, containing a known concentration of chemical, are mixed for a fixed time period (typically between 24 hours and 7 days). After the designated mixing period has elapsed, the concentration of the chemical is determined in either the solid or aqueous phase, or both. The concentration in the aqueous phase can then be compared with that in a control sample (containing no soil) to determine the amount of pollutant bound to the soil phase.

One of the most common methods used to assess movement of chemicals in soil in the laboratory is the use of some form of column chromatography.[30] This usually involves artificially packed columns of sieved soil, used in order to give a reproducible system. Water is applied to the surface either to maintain a constant head pressure, or at a rate similar to the infiltration rate in the field to avoid ponding on the surface. The chemical can then be leached immediately after application to the soil surface or following a pre-determined period of ageing. The amount of pollutant plus any degradation products in the leachate water can be determined, as well as the vertical distribution of chemical in the soil following destructive sampling of the

column. As with batch experiments, data from these studies can be used to compare leaching behaviour of different chemicals.

Unfortunately, laboratory-based methods for the estimation of chemical behaviour in the field frequently do not allow an accurate estimation of "real" behaviour in the environment. Laboratory tests of mobility can give reproducible and comparable results, but conditions in these experiments rarely simulate a normal field situation. The main problem is that the studies are usually of relatively short duration and hence only take account the simplest controlling factor - the extent of adsorption of the chemical by the soil. The batch procedure produces repeatable and widely used results, but Bilkert and Rao found that batch *Kd* values were incorrect in the calculation of the movement of two pesticides.[31] They concluded that because the system was in a dynamic non-equilibrium state, the use of an equilibrium value such as *Kd* in a pesticide transport model tended to overestimate both sorption and desorption. In addition, the use of column techniques fail to describe lateral variability in transport or, the rapid transport of water through soil macropores (the columns are uniformly packed).[32]

Field studies of pollutant transport are inherently more difficult to perform, however, they provide a much more realistic prediction of the fate of chemicals in the environment. Conventional field studies use soil column field lysimeters of undisturbed soil which are close to natural field conditions. These consist of steel columns which are driven into the soil and are installed next to a trench which allows access to holes excavated under the columns for continuous collection of soil leachate.[33] The field studies allow plants and crops to be grown on the site as normal, where pesticides and/or pollutants can be introduced to them in the usual manner. The results of most laboratory studies are dominated by adsorption / desorption relationships but in the field, degradation rates are also important, and the regular changes in both the magnitude and direction of water flow in the soil will have a highly significant effect on movement. Other advantages of field studies include proper maintenance of soil structure, the ability to sample on a regular basis, and the realistic influence of soil temperature and moisture on degradation and other loss processes.[30]

In almost all soil sorption studies, whether they be in the laboratory or the field, radiolabelled chemicals are usually chosen as the test analytes. In the estimation of the movement of organic chemicals, the analytes contain carbon-14 atoms which are beta-emitters. The molecules behave in an identical way to the normal chemicals but allow

the easy estimation of their concentration, in soil or leachate, by radioassay techniques (conventionally, liquid scintillation analysis). Usually this involves removing a known volume of leachate (which has been physically separated from the soil fraction) and preparing it for counting by adding an appropriate amount of liquid scintillant cocktail. This technique, in comparison with non-radiolabelled studies, does not require the extraction of analytes from the solid or liquid phase prior to analysis and therefore does not suffer from the problems of inefficient recovery.

There are many examples in the literature of studies involving the uptake and retention of pollutants into the soil environment. Principally, these involve pesticide adsorption since they routinely come into contact with soil during their application to pests and crops.

Early work often focused on the adsorption of organochlorine pesticides since their use peaked around the 1950's. The adsorption and movement of lindane was investigated in soils of different textures (but similar organic contents) and particle sizes.[34] Relative adsorption rates and isotherms were determined using laboratory-based batch *Kd* experiments using 2 g of soil with 20 ml of a lindane solution containing 4 ppm lindane. The results indicated that in excess of 97 % of the total lindane content existing in these soils, under saturated water conditions, would be in the adsorbed phase and that soil organic content was responsible for the high adsorption. Lindane was also the subject of a study by Adams and Li who investigated the soil properties responsible for pesticide sorption.[35] Soils were sampled from various locations and varied widely in organic carbon, collodial clay, cation exchange capacity, pH, and moisture retention properties. Variability in lindane sorption was found to be due almost exclusively to organic carbon, with the data being fit to a quadratic model using multiple linear regression. The extractability and formation of bound residues in an agricultural loam soil were investigated with so-called "non-persistent" insecticides (methylparathion and fonofos) and with "persistent" insecticides (dieldrin and pp'-DDT), all of which were ^{14}C radiolabelled.[36] The field soil (10 g) was placed in a vial and an acetone solution of the radiolabelled insecticides spiked onto the soil surface, which was kept constant at 20 % moisture (throughout the test) and left in the dark for 28 days. After the incubation period, the soils were liquid-liquid extracted three times with different polarity solvents and the solutions concentrated and counted using liquid scintillation counting (LSC). In the case of the persistent insecticides, only 6.5 % of the applied dieldrin and 25 % of the applied p,p'-DDT were found to be bound to the surface, whilst 95 % and 72 %, respectively, were recovered by solvent extraction. The results obtained from the non-

persistent organophosphorus compounds show 43 % of the methylparathion and 35 % of fonofos bound to the soil after 28 days. The results indicate that, in this particular study, contrary to the "persistent" nature of the organochlorine pesticides, very little dieldrin and p,p'-DDT were irreversibly bound to the soil.

The wide-spread use of s-triazine and urea herbicides over the last few decades has also meant that their adsorption onto soils has been extensively studied, with an early review in the subject by Hayes.[23] In more recent work, the sorption of triazines has also been linked to the clay content as well as the organic carbon content of the soil. Grundl and Small used soils which ranged from 0.4 % to 3.2 % organic carbon and clay content ranging from 24 % to 51 %.[37] Batch experiments were carried out to investigate the extent of atrazine and alachlor sorption within a 48 hour period. The results indicated that mineral phase sorption of the two compounds was significant and that sorption due solely to organic carbon, in clay-rich organic-poor soils did not occur. In a similar study, Bottero *et al.* investigated the adsorption of atrazine onto zeolites and organoclays in the presence of background organics, in an attempt to identify possible adsorbents to remove pesticides from natural water in treatment works.[38] Several different zeolites were investigated which varied in their relative hydrophobicity. Atrazine was found to have a high affinity for organics contained within the natural water, which in turn exhibited an affinity for some of the zeolites, enabling efficient removal of atrazine from waters containing organics.

In the majority of studies involving hydrophobic pesticide sorption to soil, the main soil characteristic affecting the amount sorbed is usually reported to be organic carbon content. Unfortunately, there are several accepted methods used to determine carbon content (or simply organic matter) and widely differing results have been obtained using the alternative methods as well as the same methods in different laboratories.[39] Therefore predicting the sorption of pesticides can prove difficult and is dependent on the technique used to determine the amount of organic matter present. In the above study, several ^{14}C radiolabelled urea herbicides were correlated with both soil organic matter and humic matter content, obtained using a dichromate-sulphuric acid digestion and a sodium hydroxide digestion, respectively. The results indicated that adsorption was correlated more strongly with soil humic matter than soil organic matter content.

The publications discussed are by no means an extensive review of the subject of pesticide sorption onto soils, but serve as an indication of the extensive belief that the extent of sorption is solely due to amount of humic substances present within the soil

structure and the relative hydrophobicity of the sorbent. However, as some of the studies show, this may be a generalization and many other factors may play an important role in determining the amount of pesticide adsorbed.

1.3.2 The Octanol-Water Partition Coefficient

The distribution of non-polar organic compounds between water and natural solids (soils, sediments and suspended particles) or organisms can, in many cases, be viewed as a partitioning between the aqueous phase and the bulk organic matter present in natural solids and living forms. As early as 1900, scientists studying the uptake of non-polar drugs by organisms discovered that they could use water-immiscible organic solvents like olive oil as a surrogate for organisms when assessing the accumulation of these pharmaceutically important organic molecules in the organism.[40] Although the uptake of the chemical was not identical to that of the organism it was in direct proportion to it. In the 1950's, similar work by Collander[41] favoured alcohols such as *n*-octanol as possible reference solvents because polar hydrogen-bonding solvents were best suited to model lypophilic substances reacting with biosystems. More recently, environmental researchers have used the same principles when studying the retention of organic chemicals on soil humic substances and other naturally occurring organic phases. This correlation exists because the same chemical characteristics which control the distribution of compounds between water-immiscible organic solvents and water also determine environmental partitioning from water and natural organic phases. *n*-Octanol was chosen as a suitable approximation to physiological and environmental matter because it contains both polar and non-polar functionality and therefore allows more polar molecules to be partitioned within it to some extent. Octanol-water partition coefficients, or K_{ow}, can vary over many orders of magnitude depending upon the compound studied and are therefore often expressed in logarithmic form, where they are known as Log P. K_{ow} is therefore calculated by using equation 1.2.

$$K_{ow} = \text{concentration in } n\text{-octanol / concentration in water} \qquad (1.2)$$

In estimating K_{ow} values two assumptions are made:-

1. K_{ow} is independent of the solute concentration in the aqueous phase and,
2. Organic solvent molecules present in the aqueous phase to not affect the value.

The first assumption is reasonable for most compounds since the likelihood, even at saturation concentrations, of two solute molecules positioning themselves near one another is negligible. However, the second assumption may be questioned because interactions between the solute partitioned in the aqueous phase and the organic phase also present in the aqueous phase may be significant. This is particularly true for solvents like *n*-octanol with appreciable water solubility and very hydrophobic solutes.

There are many different ways in which *n*-octanol-water partition coefficients have been estimated. Traditionally, direct measurements of K_{ow} have been carried out using conventional "shake-flask" methods where a compound is simply shaken in a vessel containing equal amounts of *n*-octanol and water and then the concentration of the compound in each solvent measured.[42] Unfortunately, this experimental approach is restricted to compounds with K_{ow} values of less than approximately 10^5, since for more hydrophobic compounds the concentration in the aqueous phase becomes too low to be accurately estimated. Other experimental techniques to calculate K_{ow} include its estimation from chromatographic retention data. Reverse-phase high-performance liquid chromatography (HPLC) has been used to generate such data by using non-polar column packing (consisting of C_8-C_{18} *n*-alkanes bonded to a silica support material) and polar mobile phases such as water and methanol. In principle the same situation is obtained as is seen in shake-flask methods where the non-polar compounds are more strongly adsorbed to the stationary phase and hence have the longer retention times. The retention times can then be correlated with compounds whose K_{ow} values are known and therefore K_{ow} values for unknown compounds interpolated. The technique has been successfully used to estimate K_{ow} values for organochlorine pesticides[43] and herbicides.[44,45]

An estimation of octanol-water partition coefficients can be obtained from structural group contributions for the molecule of interest. It was noted that specific structural units such as a methyl group always increase a compounds K_{ow} by about the same amount when they are added to a molecule instead of for example a hydrogen atom. Similarly these consistent contributions are also observed for other structural groups although they often depend upon their close environment within the molecule. In this way, even complex molecules can be broken down into their various structural sub-units and their individual effect on K_{ow} estimated. Such building blocks have been termed fragments, and the quantities that are empirically found to contribute to the overall K_{ow} termed fragment constants.[46]

A wealth of partition coefficient data for chemicals of environmental concern is now available, with a recent review on the subject of Log P values containing data on over 200 pesticides.[47] This and other reference sources are commonly used in many areas of analytical chemistry to give an indication of the relative hydrophobicity of a compound. Partition coefficients may be of particular use in organic sample preparation since they can be used to evaluate the likelihood of removal from aqueous samples as well as the degree of adsorption to soil and sediment samples and have been used extensively in this role throughout the research.

Chapter 2

Sample Preparation of Organic Pollutants in Complex Matrices

2.1 Sample Preparation of Organic Pollutants in Complex Matrices

The ubiquitous presence of many of the organic pollutants, described in chapter 1, in the environment necessitates their constant monitoring to prevent harmful contact by humans or animals. Pollutants of such nature are commonly found in greatly differing samples ranging from soil and sediments to drinking water, air, and animal tissues. Although some environmental samples are inherently ready for analysis by such instrumental techniques as chromatography or spectroscopy, the majority require removal from their environment or "matrix" in which they are found before they can be analyzed. This is partly due to most samples incompatibility with the analytical instrument used but, in environmental chemistry, also due to the analyte of interest usually being present as a minor or trace amount in a bulk sample matrix. The most common method to remove organic analytes from the sample matrix is by the use of a solvent which has some affinity for the analytes. Such a process may be deemed "extraction" if the analyte is contained in a solid matrix such as soil, or "partition" if the analyte is present in a liquid matrix, for example water or blood. In either process, the matrix is allowed to come into contact with the chosen solvent, usually combined with some means of agitation. Often the solvent is present in a much lower volume than that of the sample (particularly in aqueous samples) giving an instant concentration effect. If the chosen organic solvent does have some affinity for the analytes, favourable interactions can occur between the analyte molecules and those of the solvent, similar in nature to those discussed in section 1.2.1. As the contact time is increased between the solvent and sample, more and more analytes are removed from the matrix into the solvent until saturation occurs or strong binding to the sample matrix prevents further removal. Once the analytes have been partitioned in a suitable solvent, clean-up procedures to remove interferences and concentration by evaporation can be easily achieved. Ultimately, analyte detection, quantitation, and confirmation is routinely obtained using chromatographic techniques. The most common traditional methods of sample preparation are described in the next section.

2.2 Traditional Methods of Environmental Organic Sample Preparation

Several different solvent partition / extraction methods exist depending on whether the sample matrix is liquid or solid. Typically, organic solvents have been used for this task since they are simple to use, readily available at high purity, and often have

boiling points lower than the analytes partitioned in them, allowing easy analyte concentration by evaporation. In almost all environmental standard methods, produced by such government bodies as the US Environmental Protection Agency (EPA) or the British Standing Committee of Analysts (SCA), removal and pre-concentration of target analytes is achieved by solvent extraction. The removal of pollutants from both liquids and solids are described.

2.2.1 Liquid-Liquid Partitioning

The identification and quantification of pollutants in matrices such as drinking and surface water is required for measuring environmental pollution levels. Various pre-concentration methods based on different physico-chemical principles are used for this purpose. Of these liquid-liquid partitioning (LLP) is the most commonly used.[48]

Partition (or extraction in the case of solids) is a separation method that involves the transfer of a substance from one material phase into a second material phase. When the two phases are immiscible liquids, the method is known as liquid-liquid partitioning. In LLP a compound is partitioned between two solvents. The success of the separation depends on the difference in the solubilities of the compound in the two solvents.[49] Generally, the compound which is to be extracted is insoluble in or only partially soluble in one solvent but very soluble in the other solvent. A variety of extraction solvents are currently used for LLP including, hexane, chloroform, dichloromethane, ethyl acetate, toluene, and cyclohexane, and the choice of the best solvent depends on the analyte to be partitioned.[50]

When a solution (a solute in a solvent) is shaken with a second immiscible solvent, the solute distributes itself between the two liquid phases. When the two phases are separated again into two distinct solvent layers, an equilibrium will have been achieved such that the ratio of the concentrations of the solute in each layer defines a constant. This constant, called the distribution coefficient (or partition coefficient) K is defined by:-

$$K = C_2 / C_1 \qquad (2.1)$$

where C_1 and C_2 are the concentrations at equilibrium of the solute in solvent 1 and 2, respectively.[51] This relation is independent of the total concentration and the actual amounts of the two solvents mixed. The distribution coefficient has a constant value

for each solute considered and depends on the nature of the solvents used in each case. According to the distribution coefficient, it is obvious that not all the solute will be transferred to the second solvent in a single extraction unless *K* is very large. Usually several extractions are needed to remove all the solute from the solute matrix solvent. In partitioning a solute from a solution, it is therefore always better to use several small portions of the second solvent than to make a single extraction with one large portion.

Many of the standard sample preparative methods used for the removal of common pollutants (OCPs / PCBs, OPPs, triazine herbicides and PAHs) from water have many similar stages. However, different solvents are often more compatible with certain analytes and are therefore favoured in some methods. These include hexane for the extraction of OCPs / PCBs and OPPs from waters (book 13* and book 60*, respectively), dichloromethane for the removal of triazine herbicides from water (book 90*), and cyclohexane for extraction of PAHs (book 113*) A general protocol as published by the SCA is described.

* All Books in the reference Series from the Standard Committee of Analysts.[52]

General Liquid-Liquid Partitioning Procedure

Typically, a known volume of water (1-2 litres) is taken and 50 ml of solvent added in a 2.5 litre bottle. The bottle is then stoppered and shaken vigorously for anywhere between 5 minutes and 2 hours, or until equilibrium is said to be complete. The two immiscible liquids are then transferred to a separating funnel, where they are allowed to separate for approximately 5 minutes. During partitioning emulsions frequently form (a colloidal suspension of one liquid in another) when minute droplets of the organic solvent are held in suspension in the aqueous solution. Emulsions may require a long time to separate into two layers and are therefore problematic. Several techniques are available to break up emulsions. Usually if one of the solvents is water, a saturated solution of sodium chloride is added which helps destroy the emulsion by making the aqueous and organic layers less compatible (the mutual solubility in the solvents causes the emulsion). However, if a sample is known, through prior experience, to form emulsions, the mixing should be gentle and non-vigorous. When good separation is achieved, the aqueous phase is run-off. At this stage fresh solvent may be added and the extraction repeated as many times as required to ensure near quantitative removal of target analytes. The two solvent portions are then combined and then must be dried since after an organic solvent has been shaken with an aqueous solution it will be "wet", that is, it will have dissolved some water even though its

miscibility with water is not great. The amount of water dissolved varies and depends upon the organic solvent. To remove water from the organic layer, a drying agent is used which is typically an anhydrous inorganic salt that acquires waters of hydration when exposed to moist air or a wet solution. Anhydrous sodium sulphate crystals (which have been heated to high temperatures, often exceeding 400 °C, for several hours to remove any inherent moisture) are commonly used as drying agents and are packed into a column through which the wet organic solvent is passed. The dried extract, which will be approximately 100 ml in volume, must then be concentrated to allow detection limits to be met. Due to the volatile nature of many organic solvents, this is accomplished by evaporation. Different apparatus can be used to effect the evaporation although the Kuderna-Danish evaporator is perhaps the most well known. Further concentration is performed in a warm water bath (<40 °C) under a gentle stream of nitrogen, where care must be taken not to lose any volatile analytes. Typical end volumes are approximately 1 ml which are sufficiently concentrated to undergo chromatographic analysis.

There are many variations in this general procedure depending on the analytes of interest and the specific aqueous matrix. These include changes in solvent, pH, and ionic strength. Often intermediate clean-up stages are incorporated prior to the solvent drying. An example of this is the removal of PCBs from OCPs on a silica gel column since they interfere with the subsequent OCP analysis using gas chromatography (GC) with electron-capture detection. In addition, during the concentration stage, the extraction solvent is frequently switched to a solvent more compatible with the chromatographic analysis technique being used. OCP / PCB extraction may be used as another example if dichloromethane is used as the extracting solvent since this is not compatible with an ECD detector. Usually, the dichloromethane extract is evaporated to dryness under nitrogen and switched for a different solvent which does not contain halogens (typically hexane).

2.2.2 Solid-Liquid Extraction

Many pollutants which are found in the environment are relatively non-polar and are therefore likely to be found in matrices containing substantial amounts of lypophilic material. Typical matrices include soils and sediments as well as the fat tissues of animals. Thus a great deal of environmental interest is directed towards the analysis of these solid materials to monitor for the presence of xenobiotic pollutants. As with liquid samples, pollutants in solid matrices are not directly compatible with the

majority of analytical instrumentation and therefore require some form of sample preparation. Again in common with liquid samples, solids are usually mixed with an organic solvent which has a certain affinity for the target analytes. Unfortunately, unlike the partitioning of non-polar analytes from an aqueous sample into a non-polar solvent, extraction of these analytes from solid samples is inherently more difficult. This is primarily due to sorption of the analytes to the solid which is in direct competition with their removal using solvent extraction. Full recovery of analytes may therefore take much longer to complete (or may not be possible), with extraction of solids often requiring several hours or even days to accomplish. In addition, solid samples are far more "dirty" and usually contain much more co-extractable material when compared to aqueous samples. This material ranges from fats and other lypophilic substances in animals tissues to simple particulate matter which can be transferred from soil samples. The sample preparation of solid matrices is therefore likely to require some form of clean-up procedure before analysis can take place, adding to the length of the overall procedure.

There are several methods by which solid samples are extracted using organic solvents. However, by far the most common is the use of Soxhlet apparatus or by sample agitation using sonication.

2.2.2.1 Soxhlet Extraction

The extraction of solid materials by organic solvents in a Soxhlet apparatus is the most widely used technique for the concentration of non-volatile and semi-volatile target analytes in solid samples. In fact, the procedure has become so widely recognized that all other extraction methods from solids have their performance routinely assessed against that of Soxhlet extraction. Almost all of the US EPA methods for the analysis of pollutants in solid samples describe the use of a Soxhlet extraction in the initial sample preparation stage, with method 3540* detailing the Soxhlet procedure. The initial Soxhlet extraction is then used in the gas chromatographic US EPA methods for the analysis of organochlorine pesticides and PCBs (method 8080*), PAHs (8100*), organophosphorus pesticides (8140*), and chlorinated herbicides (8150*).

* All of the methods are obtained from the US Environmental Protection Agency.[53]

The Soxhlet extraction is named after the inventor of the apparatus, Franz Soxhlet, a German food chemist.[54] The modern apparatus is made from glass with QuickFit joints and is used with a round-bottomed flask and condenser. A schematic of the apparatus is shown in figure 2.1. The solid sample which is to be extracted is placed in a porous thimble inside the Soxhlet apparatus. The thimble allows the passage of solvent but not the sample and therefore prevents particulate matter being transferred into the extracting solvent. The solvent (enough to completely fill the chamber in the Soxhlet apparatus containing the thimble) is then added to the round-bottomed flask and the condenser fitted to the top of the apparatus.

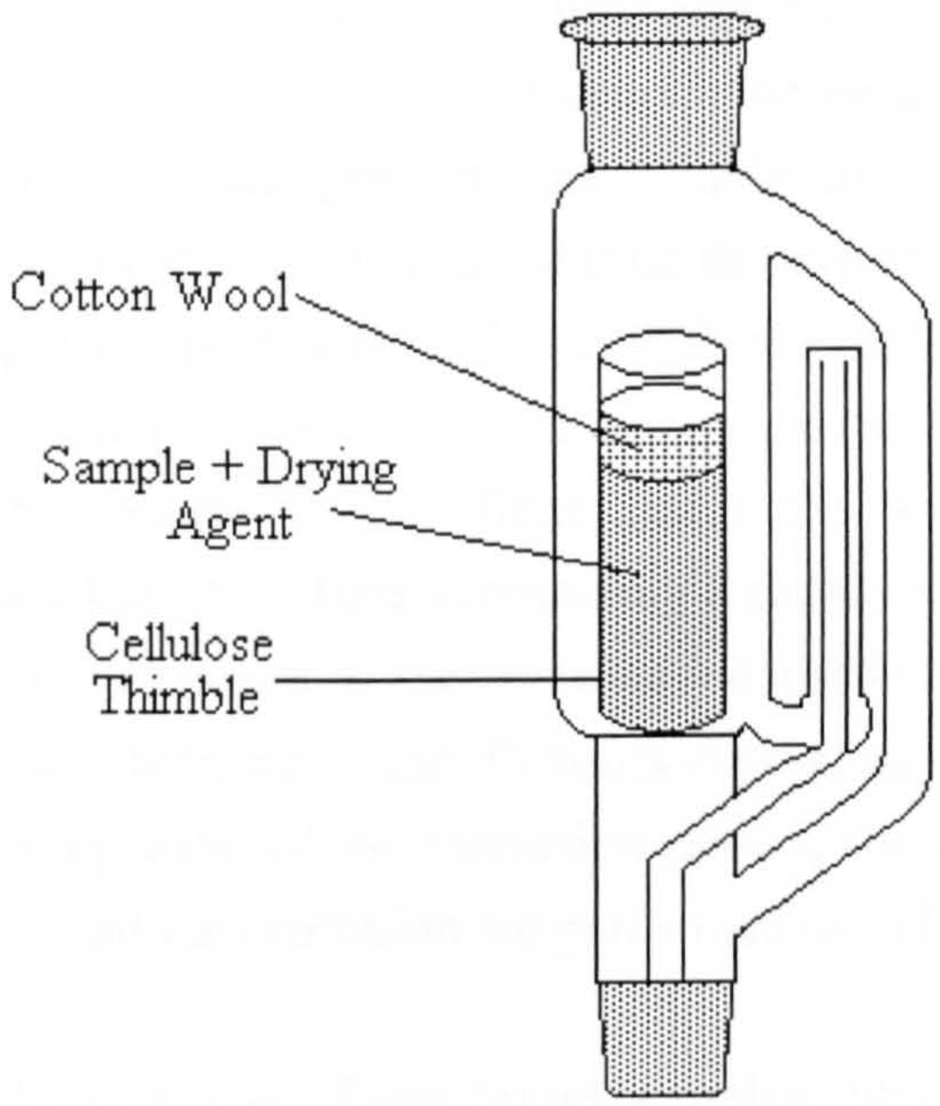


Figure 2.1 Schematic of a Soxhlet Apparatus.

As the solvent is heated, vapour is produced which passes through the outer side-arm where it is eventually turned back to liquid in the condenser. This liquid then falls back into the chamber where it comes into contact with the sample. As more vapour is condensed, the chamber is filled until the level of liquid reaches the top of the inner side-arm. Upon reaching this, the solvent is siphoned back into the round-bottomed flask, whereupon the whole procedure is repeated. However, because the extracted analytes usually have far higher boiling points than the solvent, they are retained in the flask during heating and therefore the thimble is always washed with clean solvent. This prevents re-adsorption of the analytes back onto the sample matrix. The heat applied to the solvent dictates the amount of vapour produced and therefore the speed at which the chamber is filled. Typical rates of filling and emptying are

approximately 6-8 cycles per hour. Thus, a sample which is extracted for 6 hours can expect to be washed with fresh solvent around 40 times. The general US EPA procedure for the sample preparation of non- and semi-volatile analytes from solid samples is described below.

General Soxhlet Procedure

Samples (typically soils or sediments) are left to dry naturally as much as possible before discarding any foreign objects such as sticks, leaves and rocks. The samples are then ground (either by hand or using a mechanical grinder) so that the particles pass through a 1 mm sieve. A portion of the solid sample (commonly 10 g) is mixed with an equal portion of a drying agent (anhydrous sodium sulphate) which removes any residual moisture and allows intimate contact with the sample and the organic solvent. In addition, the drying agent prevents the sample from being compacted which facilitates the free draining of the thimble during the extraction period. The mixture is then placed in the porous thimble (usually made from cellulose) and a glass wool plug (both of which have been pre-extracted with the organic solvent) inserted at the top to prevent escape. The thimble is then fitted inside the Soxhlet apparatus which is attached to the round-bottomed flask (containing a greater volume of solvent than the volume of the thimble chamber in the apparatus) and condenser. The sample is then extracted for anywhere between 6 and 48 hours depending on the target analytes and the matrix. After completion of the extraction period, the extract is allowed to cool and drying, clean-up and concentration are performed as in liquid-liquid partitioning.

As in the removal of analytes from liquid samples, variations exist between this general method and the exact procedure employed for specific compounds. Again solvents are chosen for their affinity for the target compounds as well as their compatibility with the subsequent analytical detection method. However, probably the most widely differing variable is the time of extraction since it remains difficult to estimate the time required to effect quantitative removal of target compounds.

2.2.2.2 Extraction by Sonication

The second most utilized technique for extracting organic compounds from solid samples (although less prominent than Soxhlet) is that of extraction enhanced by sonication. Often publications compare the performances of Soxhlet and sonication for the removal of compounds from various solid matrices (for example[55]).

Ultrasonic sound waves are those having frequencies above the audible range, that is, above 20,000 Hz. There are numerous applications of ultrasonics which are conveniently split into low- and high-amplitude areas. Low-amplitude applications include sonar as an underwater detection system and detective devices to determine the presence of flaws in solid materials. However, the use of ultrasonics in extraction may be classified as a high-amplitude application. When a liquid is subjected to high-amplitude acoustic waves they can rupture the liquid, resulting in the formation of gas- and vapour-filled bubbles. When such a cavity collapses, extremely high pressures are produced, by the process called cavitation, which has a number of mechanical and chemical uses. These include ultrasonic cleaning and degassing of liquids, although it is the use of ultrasonics in the effective dispersion of solids in liquids which is used to enhance the extraction of target compounds from solid samples.[56]

General Sonication Procedure

The details of a standard method for extracting non- and semi-volatile organic compounds from solid matrices are given in the US EPA method 3550.[53] The method is divided into two sections, based on the expected concentration of the organics in the sample. The low concentration method (of use with individual organic components of ≤ 20 mg kg^{-1}) uses a larger sample size and a more rigorous extraction procedure. The high concentration method (individual organic components of ≥ 20 mg kg^{-1}) is much simpler and therefore faster.

Low Concentration Method Approximately 30 g of sample (with all extraneous material removed) is weighed into a 400 ml beaker. Non-porous or wet samples that do not have a free-flowing sandy texture are ground (<1 mm) and are mixed with double the amount of anhydrous sodium sulphate, until free-flowing. 100 ml of 1 : 1 dichloromethane : acetone is then immediately added and the bottom surface of the ultrasonic probe tip is placed approximately 2 cm below the surface of the solvent, but above the sediment layer. The sample is then sonicated for 3 minutes where upon it is decanted and filtered using vacuum filtration. The extraction is repeated two or more times with additional portions of dichloromethane : acetone and the extracts combined. Clean-up and concentration are then effected using the same techniques as described in the Soxhlet extraction.

High Concentration Method This method is similar to the above except only 2 g of sample is required to meet the detection limits and therefore only 10 ml of solvent is used.

The main advantage sonication has over Soxhlet extraction is the time required to complete. However, as with the Soxhlet method, this will vary depending on the sample and the target analytes to be extracted. Unfortunately there are also several disadvantages in that equipment costs are much higher than in Soxhlet extraction and although Soxhlet is time-consuming, the apparatus may be left during the extraction. With sonication, since the extraction repeats are relatively close together, the analyst must spend time with the apparatus overseeing the extraction.

2.3 Modern Methods of Environmental Organic Sample Preparation

In recent decades, there have been great advances in instrumental techniques for organic analysis, particularly in the areas of chromatography and spectroscopy. It is therefore unusual that although modern chromatographic techniques have become so highly developed they can separate, identify, and quantitative many components within a sample, analytical chemists still rely on the sample preparative techniques described in section 2.2, which were used when the Russian botanist Tswett first reported chromatography in 1903.[57]

An ideal extraction method should be simple to perform, rapid and inexpensive. Additionally, it should give a quantitative recovery of the analytes of interest, without loss or degradation and lead to a sample that is immediately suitable for the appropriate analysis technique without necessitating subsequent concentration or clean-up stages.[58] Unfortunately, extraction with organic solvents frequently does not meet these criteria. Many of the traditional sample preparative techniques require several hours or even days to complete and even then do not result in quantitative recovery of target analytes. Organic solvent are inherently "non-selective" in their removal of organic compounds from sample matrices, and will not only extract target analytes but any other organic compounds which are present. This may cause additional problems with co-extractives interfering in subsequent analysis. The solvent extraction protocols, as well as being time consuming, are difficult to automate meaning that time is spent performing relatively mundane tasks, further reducing the laboratory throughput capacity. During partition from aqueous samples, formation of emulsions is common which causes problems in separating the two liquids. Also, at the end of the procedure, the analyst is often left with a relatively dilute extract which requires further time to concentrate.

In addition to the problems already outlined, recent concern about the hazardous nature of many commonly used solvents, the high costs of solvent waste disposal, and the emission of hazardous chemicals into the atmosphere from evaporation (used in sample pre-concentration) have led to a move towards alternative extraction methods. Typical solvents include volatile compounds such as dichloromethane, considered to be "probably carcinogenic to humans",[59] and now found to be ubiquitous in the atmosphere.[11]

Within the last ten years, several different techniques have been introduced which resolve many of the problems inherent in organic solvent sample preparation. These novel techniques have a variety of applications with different sample matrices, and analytes are often better suited to one particular technique. However, all of the modern methods described have one feature in common in that they reduce or completely eliminate the use of organic solvents.

2.3.1 Supercritical Fluid Extraction

The recent problems associated with organic solvent extraction, particularly from solid samples, has led to the development of techniques which utilize the unique solvation properties of supercritical fluids. Supercritical fluids were first discovered in the late 1870's by Hogarth and Hannay,[60] although the potential of these fluids remained unrecognised for many years. The solvent properties of "liquefied gases" were later investigated and an extensive collection of ternary phase diagrams for liquid carbon dioxide with both organic and inorganic compounds, together with estimated solubilities of over 200 compounds in CO_2 compiled.[61] The first serious analytical application of supercritical fluids came in the early 1960's when supercritical fluorocarbons were used as a mobile phase in chromatography for the separation of a mixture of porphyrins, previously inseparable by gas chromatography (GC).[62] The development of supercritical fluid chromatography (SFC) was necessitated because one of the other main chromatographic techniques, GC, was incapable of analyzing thermally labile or reactive compounds. The use of SFC was found to be suited to such analytes since it was performed at low temperatures. However at the time, SFC did not reach its full potential as an analytical tool because the packed columns used did not allow sufficient separation and resolution. In addition, advances in liquid chromatography occurred at a similar time and much of the research into separative techniques was directed towards this promising area. Interest in supercritical fluids declined over the next two decades, until research

groups of Novotny and Lee successfully introduced capillary SFC in 1981.[63] This resulted in a greater acceptance of the technique and the availability of commercial instrumentation by the mid-1980's.

Publications as early as 1976 demonstrate the use of supercritical fluids for extraction on an analytical-scale.[64] By contrast, supercritical fluids were used much earlier in chemical engineering situations, where as early as 1936, propane at or near its critical point was used to refine lube oils (propane deasphalting) and for the purification and separation of vegetable and fish oils (Solexol process). More recently supercritical CO_2 has been used for the removal of caffeine from coffee and for the treatment of hops.[65] Commercial instrumentation became available for analytical-scale supercritical fluid extraction (SFE) around the same time as that of SFC and has all but superseded the use of "home-made" instruments in recent years. In fact, it is the advent of such modern instrumentation, combined with the need to reduce the use of organic solvents that has fuelled the current resurgence in the use of supercritical fluids in the laboratory.

2.3.1.1 Theory

Physico-chemical Properties of Supercritical Fluids

A supercritical fluid (SF) is neither a gas nor liquid but possesses properties of both. It is a gas in that it fills and takes the shape of its container (unlike a liquid), but however, has the typical density of a liquid. The definition can be best visualized by reference to the typical pressure-temperature phase diagram for a single substance shown in figure 2.2. The diagram shows the temperature and pressure regions where the substance occurs as a single phase (solid, liquid or gaseous). Such regions are bounded by curves indicating the co-existence of two phases, which are involved in sublimation, melting and vaporization. The three curves intersect at the so-called "triple point" (TP), where the solid, liquid and gaseous phases exist in equilibrium.[66]

As the vaporization co-existence curve is followed upwards, the pressure and temperature of the substance are increased. An increase in pressure makes the substance more dense and therefore more "liquid-like", whereas a temperature increase tends to make the substance less dense and thus more "gas-like" until the critical pressure (P_c) and temperature (T_c) of the substance are met at the critical point (CP). At this point the densities of the two phases become identical and the liquid and gaseous phases become indistinguishable. The substance can no longer be called

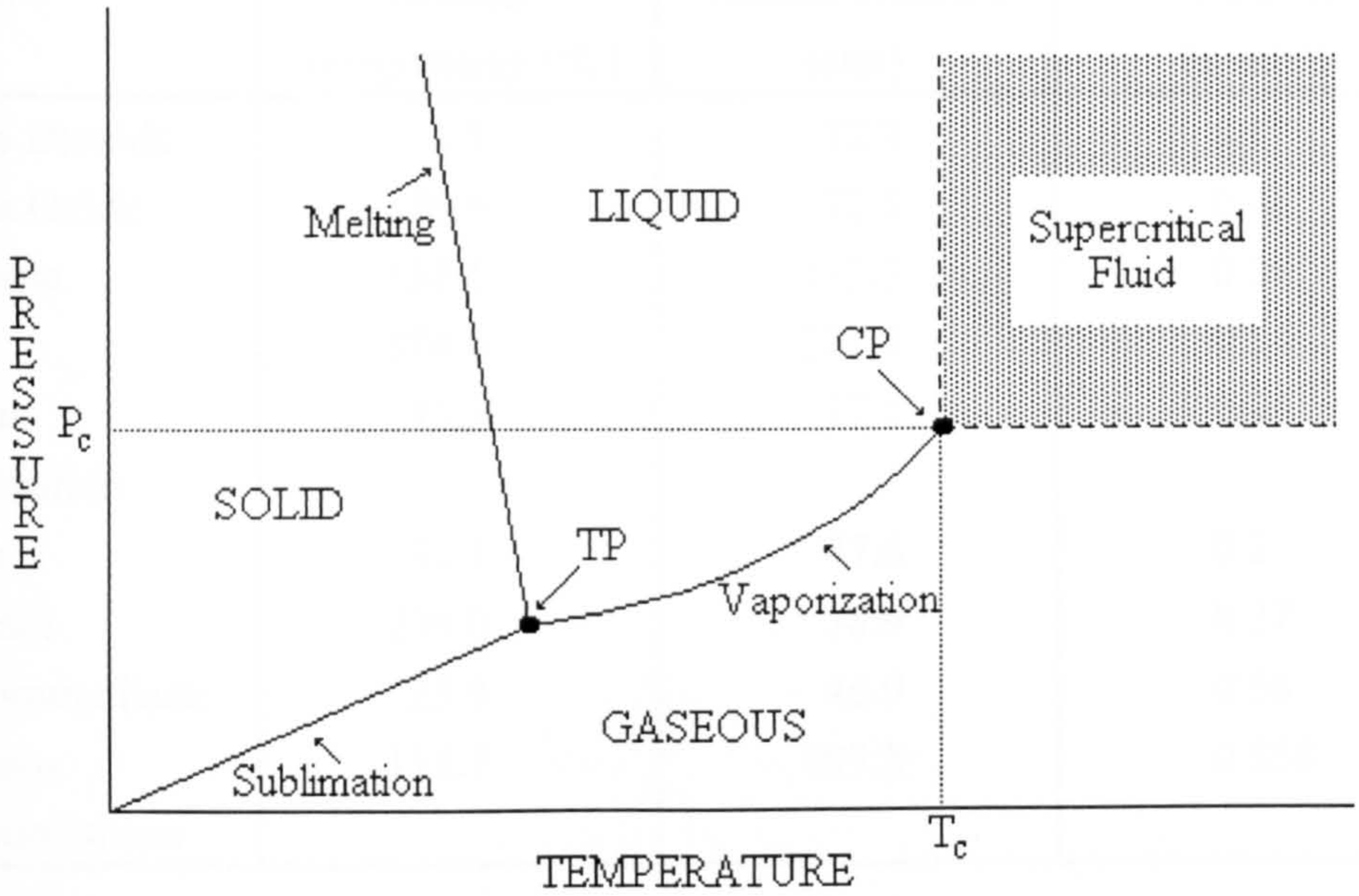


Figure 2.2 Typical Phase Diagram for a Single Substance.

liquid or gaseous and is therefore called a (supercritical) fluid. Above the CP no liquefication can take place on raising the pressure and no gas will be formed on increasing the temperature. Therefore another definition of a supercritical fluid is one that is above its critical pressure and temperature. The critical pressure and temperature are often used to characterize a supercritical fluid, with the critical values of some common solvents shown in table 2.1 along with their critical density (the fluid density at the critical point).

Even though supercritical fluids offer no advantage over liquids in terms of dissolving power, there are several physical properties of supercritical fluids which make them unique as extraction solvents or as a mobile phase in chromatography. Such significant properties as diffusivity, viscosity, and density in the supercritical region lie between those of liquids and gases, which can be used to exploit the advantages of both in the applications above.

Supercritical viscosity values lie between those of liquids and gases, giving supercritical fluids good flow characteristics. They also possess a very low surface tension that allows them to penetrate non-porous solids and packed beds much more readily than liquids. In addition, the diffusion coefficients of solutes in supercritical fluids have values between those attained in gaseous and liquid solvents. The high

Solvent	Critical Temperature (°C)	Critical Pressure (atm)	Critical Density (g ml^{-1})
Carbon Dioxide	31.3	72.9	0.47
Nitrous Oxide	36.5	72.5	0.45
Ammonia	132.5	112.5	0.24
Water	374.0	227.0	0.34
Sulphur Hexafluoride	45.5	37.1	0.74
Ethane	32.3	47.6	0.2
Methanol	239.0	78.9	0.27
Trifluoromethane	25.9	46.9	0.56
Dichloro-difluoromethane	111.7	109.8	0.558

Table 2.1 Critical Parameters of Various Common Solvents.[67]

diffusion coefficients combined with their low viscosity mean that mass transfer rates into the bulk fluid, in substances above their critical point, will be significantly higher than those in liquids. The density of the supercritical fluid depends on the pressure and temperature to which it is subjected, although it is always near to the typical values for liquids. This relatively high density (when compared to that of gases) is the basis for the good dissolving properties of supercritical fluids, where interactions between the fluid and solute molecules are quite strong. Indeed, a unique property of supercritical fluids for extraction purposes is the ability to adjust the "solubilizing power" primarily *via* mechanical compression (and additionally *via* temperature). By such means, the density of the extracting fluid can be controlled, which gives an approximate measure of intermolecular attraction, thereby providing the possibility of using one supercritical fluid to extract a range of analytes of varying polarity and molecular size. However, an increase in fluid density, in addition to enhancing the dissolving power of the fluid, also has the detrimental effect of decreasing the diffusion coefficient and increasing the viscosity. Therefore as the density is increased the effectiveness of the supercritical fluid is reduced, thus fluids should be used at the optimum density to solubilize a particular solute and not be subjected to unnecessarily high densities. The physical properties of supercritical carbon dioxide are compared to those of gases and liquids in table 2.2.[68]

All of the physical properties discussed show the greatest changes at or near the critical point for the supercritical fluid. Consequently, when utilizing the properties to

	Density ($g\ ml^{-1}$)	Viscosity ($g\ cm^{-1}\ s^{-1}$)	Diffusion Coefficient ($cm^2\ s^{-1}$)
Gases	$(0.1\text{-}2) \times 10^{-3}$	$(1\text{-}3) \times 10^{-4}$	0.1-0.4
Supercritical CO_2*	0.47	3×10^{-4}	7×10^{-4}
Liquids	0.6-1.6	$(0.2\text{-}3) \times 10^{-2}$	$(0.2\text{-}2) \times 10^{-5}$

Table 2.2 Comparison of the Properties of Supercritical CO_2 and those of Ordinary Gases and Liquids.
*Supercritical CO_2 at its critical point.

enhance extraction capabilities, the most significant response is shown near a pressure and temperature close to CP. Therefore, in the case of density, increasing the pressure above P_c has less and less effect on fluid density and thus on the extraction characteristics of the fluid.

It is these three physical properties of supercritical fluids (viscosity, diffusion coefficient, and density) that make them such excellent extracting solvents. An ability to initially penetrate non-porous material, combined with densities similar to those of liquids (giving good solute solubility) and high mass transfer rates once the solute has been dissolved all lead to the possibility of a rapid, complete extraction.

There are many substances which possess critical parameters that are obtainable in the laboratory. Some of the more common solvents used in SFE are given in table 2.1. However, high pressures and temperatures are difficult to work with in the laboratory and many of the listed fluids would not be suitable for practical extractions due to their unfavourable physical properties, costs, or reactivities. Examples include, N_2O which has been successfully used as an extracting fluid (see section 2.3.1.3) but exhibits a high reactivity towards other compounds and is dangerous if improperly used. Other fluids, like fluoroform (CHF_3), have the ability to form hydrogen bonds with certain compounds which can greatly enhance their solubility, but the high cost of the fluid limits its use in routine SFE.

By far the most widely used extraction fluid has been CO_2, which possesses a relatively low critical pressure and low critical temperature making it an ideal choice for extracting thermally labile compounds. Also, CO_2 is gaseous at room temperature and pressure allowing extracted compounds to be easily removed from solution by depressurization. In addition, CO_2 is non-toxic and provides an extraction

environment free from oxygen thereby limiting the potential for oxidizing extracted solutes leading to a low reactivity. CO_2 is available at high purity and at a low cost and is non-polar which makes it ideal for extracting non- and moderately-polar analytes. However, in general, large polar compounds exhibit almost no solubility in supercritical CO_2, even at high density because of the lack of a permanent dipole in the extracting fluid. The other fluids listed in table 2.1 have been used to extract polar analytes, since many have a permanent dipole moment, but the most common method for enhancing the solubility of polar compounds in CO_2 is by the addition of a polar co-solvent to the fluid or sample.

Polar co-solvents usually cannot be used as pure supercritical solvents because their critical temperatures are too high for thermolabile substances, although the addition of small amounts (usually ≤ 10 %vol) of co-solvents, called modifiers or entrainers, in a primary supercritical solvent (*i.e.* CO_2) can have a profound effect on the dissolution of previously insoluble compounds in supercritical fluids. The presence of a modifier does not increase the polarity of a fluid as this is determined by the dipole moment of the fluid. Such an increase arises from interaction between the solute and modifier in the supercritical fluid through preferential intermolecular forces such as those involved in hydrogen bonding. However, a modifier can affect a supercritical fluid-solute system in other ways. The increased solvating power of many systems is as a result of the increased density of the solvent mixture compared to that of the pure fluid. Also, polar co-solvents can effectively compete with analyte bonding sites on solid matrices, or help to swell the matrix allowing target compounds to be more easily removed and therefore solubilized. Water is found to effectively swell solid matrices and has been used in conjunction with methanol to successfully remove herbicides from plant and clay materials.[69]

The most common supercritical fluid entrainer has been methanol, although many other solvents have been used including other higher alcohols, and more polar compounds such as triethylamine. When methanol (or other entrainers) are added to supercritical CO_2, the critical properties of the fluid are altered and an increase in pressure and/or temperature may be required to keep the fluid supercritical. For example, with methanol-CO_2 mixtures at 50 °C there is only one phase above 95 bar whatever the composition, but below this pressure, two phases can occur. The situation is best represented by a phase diagram for the binary system, shown in a three-dimensional figure, whose axes are pressure, temperature and mole fraction. If the temperature is constant, a cross-section through such a phase diagram is a two-dimensional pressure- mole fraction plot and is shown in figure 2.3.[70]

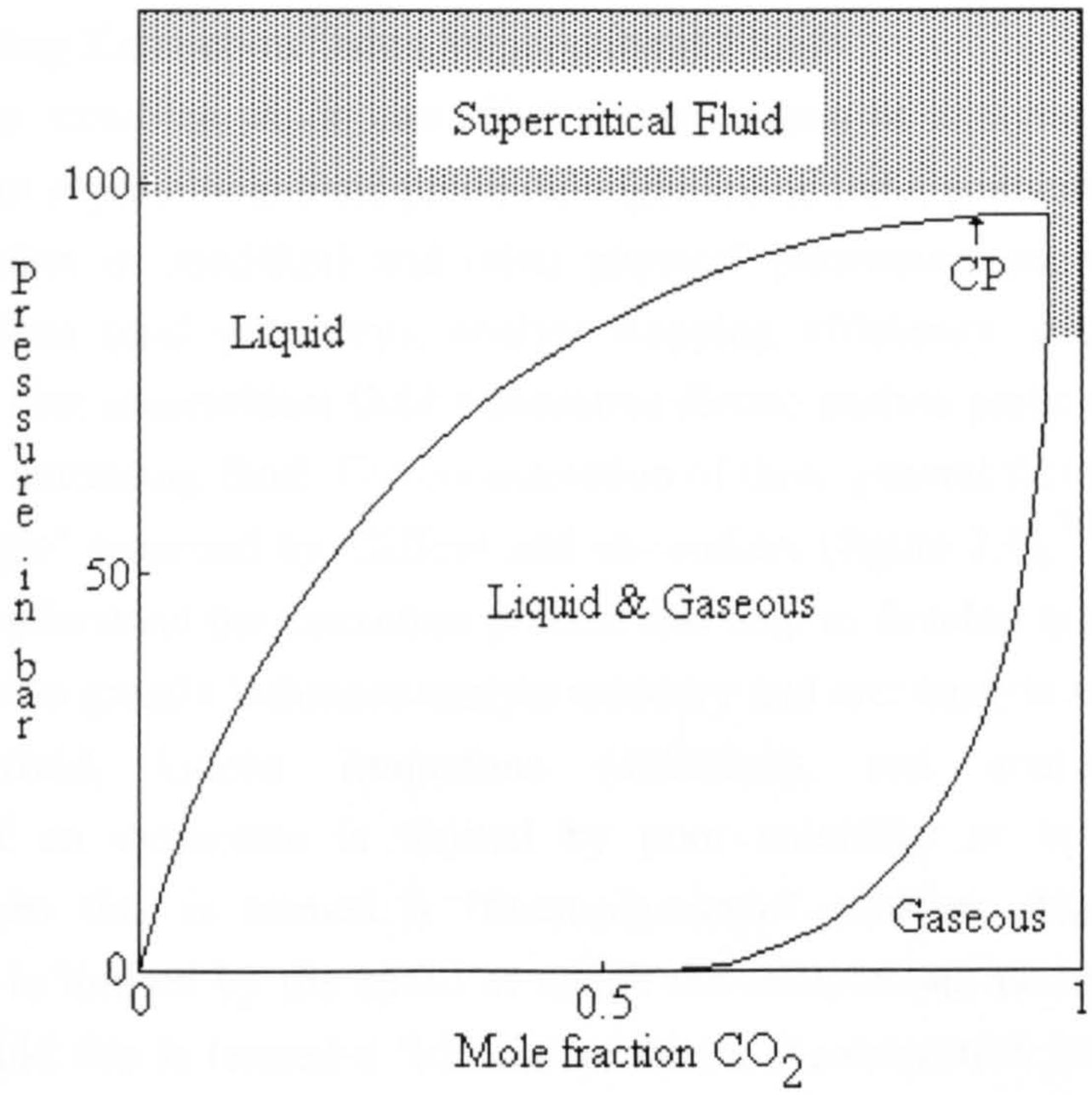


Figure 2.3 Phase Diagram for Methanol-CO_2 at 50 °C.

The diagram shows that at low pressures (not used by SFE) a single gaseous phase exists for all compositions. At high mole fractions of CO_2 the mixture is gaseous, whereas at low CO_2 mole fractions the mixture is liquid and at intermediate mole fractions, two phases exist. This two-phase region reaches a flat maximum at the critical point, CP (at 50 °C). An increase in the pressure above this point then produces supercritical conditions. Thus for the mixture to be supercritical, the system must be above 95 bar at this particular temperature. However, when the proportion of modifier is small (as with SFE), part of the gaseous phase is often considered as "supercritical" as the pure gaseous component is above the critical pressure and temperature. Hence, the shaded area in the figure is that loosely called supercritical. In addition, many of the advantages of supercritical fluids are possessed by liquids that are just sub-critical, and sub-critical fluids are often used in industrial situations. Although SFE is carried out in supercritical, one-phase conditions (because of experimental inconsistencies), it may be possible that two-phase extraction systems have an advantage in terms of agitation of the matrix being extracted.

There have been many fundamental studies on the effect of modifiers added to supercritical CO_2. The investigations include work in both the field of SFC where adsorption isotherms have been determined,[71,72] as well as SFE where the effect modifiers have on the solubility of analytes in supercritical fluids has been studied.[73]

Factors Affecting Extraction Using Supercritical Fluids

There are many variables which can affect the extraction of analytes in SFE. They include different supercritical fluid parameters (choice of fluid, pressure, temperature, flow-rate, addition of modifier) and other physical parameters such as extraction vessel dimensions (and geometry), analyte trapping efficiency, and sample pre-treatment. The first supercritical fluid parameters dictate analyte partitioning from the matrix into the extracting fluid. The consideration of three general factors, depicted in the "SFE triangle" proposed by Clifford and co-workers (figure 2.4),[70] are useful for attempting to understand the extraction process and help to develop quantitative SFE. The factors shown greatly influence analyte recovery and are: analyte solubility in the supercritical fluid, kinetic limitations (diffusion), and analyte-matrix-fluid interactions. If an extraction is limited by poor solubility or by solute-matrix interactions then this is termed a "thermodynamic" problem. However, if the extraction rate is limited by the speed at which the analytes are partitioned into the supercritical fluid this is termed a "kinetic" problem, the extraction being limited by either diffusion in the matrix or slowed by desorption kinetics.

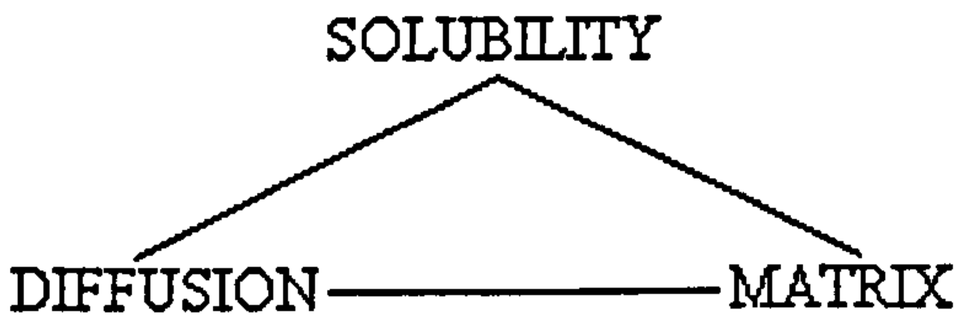


Figure 2.4 The SFE Triangle.

Solubility The first and most obvious requirement of a successful SFE extraction is the ability of the extracting fluid to solvate the target analytes. This factor is especially important at the beginning of an extraction, when extraction is occurring at a higher rate. However, a variety of other solute-supercritical fluid interactions are possible ranging from physical sweeping to chemical reaction.

The solubility of compounds in solvents such as CO_2, below their critical point, is determined by the vapour pressure of the solute, since CO_2 is a relatively ideal gas with no special solvent properties under these conditions. As the pressure is increased, the fluid density also increases up to a point where the solubility of analytes is greatly enhanced. As the fluid density increases, the mean intermolecular distance decreases and specific interactions between solvent and solute increase proportionally. Near the

critical pressure of the fluid, the solubility increases sharply as a result of the marked increase in density with pressure. At extremely high pressures, the solubility reaches a maximum until further increases in pressure cause the solubility to decrease as a result of repulsive forces "squeezing" the solute out of solution. As pressure is raised, the onset of solubility occurs at a particular value for a solute-solvent system termed the "threshold pressure", which was first introduced by Giddings.[74] Above this pressure, a compound will have a measurable solubility in the supercritical fluid and although of use in choosing a starting pressure for SFE, the value of the threshold pressure is technique-dependent and will vary slightly, depending on the sensitivity of the analytical method chosen to monitor the solute concentration in the supercritical fluid phase.

Temperature also has an affect on the solubility of an analyte in a supercritical fluid, although this may be more difficult to estimate. At constant pressure, solubility has a minimum value for a particular temperature. As low temperatures are increased, solubility falls because, as the temperature rises, the density and therefore the solvating effect is reduced. However, as further increases are made, the volatility of the solute also increases, and eventually this effect exceeds the effect of the reduced density and the solubility rises.

In industrial supercritical fluid applications, where extraction is being carried out on a large scale, optimization of solubility is important because of high running costs. However, for quantitative analytical purposes, it is not important that the solute is extremely soluble, as extraction is carried out to exhaustion, by which time solubility is a less important factor (although, good solubility speeds up the initial rate of extraction and therefore reduces the overall extraction time).

As mentioned previously, 'CO_2 is a non-polar molecule and therefore good at dissolving non- and moderately-polar molecules. However, this is a simple generalization and a knowledge of analyte solubility in supercritical CO_2 may greatly reduce the initial time required to develop a SFE method. To this end, there are several methods for determining solute solubility in supercritical fluids. Perhaps the most simple technique, although also the longest to perform, is the use of SFE equipment to "extract" the pure solute. The extraction is carried out at constant temperature and pressure, and at relatively low flow-rates to ensure that the fluid is saturated on exit. The extract is then collected for a set time period, where upon it is analyzed to obtain the amount extracted. Often gravimetric methods are used to obtain the amount of solute extracted but require large amounts of solute (a problem for toxic

or high-cost compounds) and lack the necessary sensitivity for low-solubility analytes. By calculating the number of moles of solute extracted as well as the number of moles of solvent (from the flow-rate and the density), the mole fraction can be estimated. If the experiment is repeated for different pressures (at constant temperature), solubility isotherms are produced which graphically show the effect of pressure on solubility, allowing the threshold pressure and the maximum solubility pressure to be estimated. This general technique has been used to estimate the solubility of pesticides in supercritical CO_2,[75,76] cholesterol in supercritical ethane[77] and more recently, PAHs using an on-line arrangement.[78]

Despite the extensive use of experimental methods to determine solute solubility in supercritical fluids, they are time-consuming and are dependent on the analytical technique used to calculate the amount of solute extracted. In light of this, several techniques have been proposed to predict analyte solubility from a knowledge of the physical properties of the particular solute and solvent. The pressure at which a solute attains its maximum solubility in a compressed fluid was estimated by King[79] using the equation proposed by Giddings[74] which relates the Hildebrand solubility parameter of the gas to its critical and reduced properties. When the solubility parameter of the extracting fluid is equivalent to that of the solute, maximum solubility should be attained. The relationship was used by King to estimate the supercritical fluid density required for the maximum solubility of lipid phases in supercritical CO_2 and by Kane *et al.* to predict the solubility of analytes based on both their hydrophobic interaction (calculated from Log P values) and the solubility parameter.[80]

Other methods have been used which require more information about the solute to predict solute solubility in supercritical fluids. An equation of state such as the Peng-Robinson equation was used by Bartle *et al.* to predict the solubility of pollutants, including PAHs, in supercritical CO_2.[81,82] The Peng-Robinson equation of state may be used to correlate data over a limited range of temperatures and pressures but requires many constants to be known for the solute, some of which may only be obtained by experimentation. In addition, the calculations required are relatively complex and errors easily occur, resulting in its lack of suitability for use in the day-to-day running of SFE in the laboratory.

Kinetic Limitations Extraction by a supercritical (or any) fluid is never complete in a finite time. Instead, it is relatively rapid initially, but there follows a long tail in the curve of percentage extracted versus time, as shown in figure 2.5. As mentioned

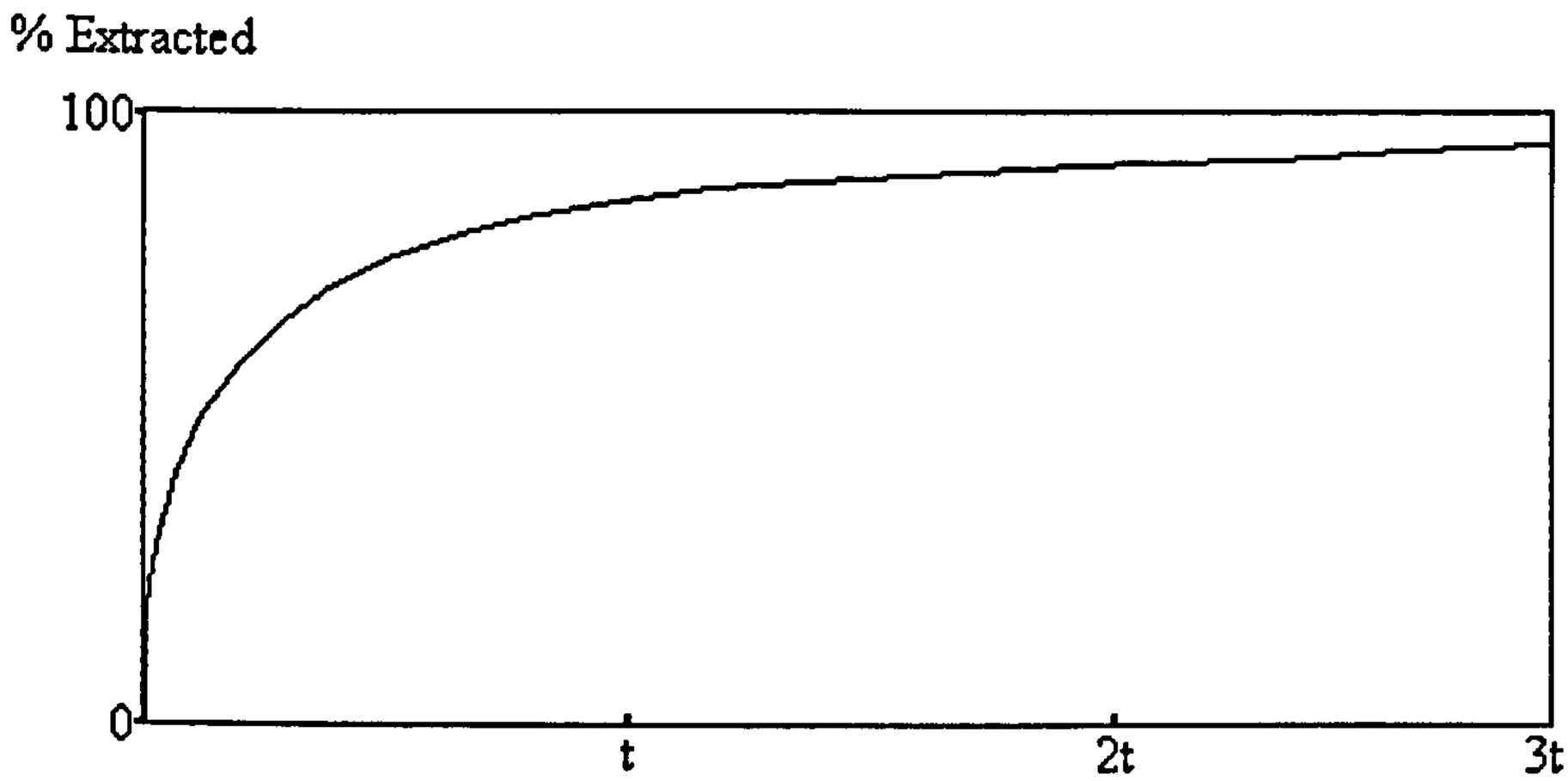


Figure 2.5 General Extraction Curve of Percent Extracted *versus* Extraction Time.

previously, the initial part of the extraction (time <t) is controlled by the solubility of the solute in the supercritical fluid. After a finite time, the extraction curve starts to become convex with respect to the time axis as the extraction experiences a transition from solubility to diffusion controlled kinetics. SFE is a more rapid extraction technique as opposed to extraction using liquid solvents because of the high diffusion rates of solutes in supercritical fluids. Although solute diffusion through supercritical fluids is rapid, once analytes which are weakly bound to the surface of the matrix have been solubilized and removed, the rate of the latter part of the extraction is dependent upon the diffusion of the analytes through the sample matrix. Again the low viscosity and surface tension of supercritical fluids aids their passage through solid samples when compared to that of liquid solvents but slow desorption through complex matrices, such as those containing water, hinder rapid extraction even with supercritical fluids. The situation is illustrated in figure 2.6 for extraction from solid matrices, where there are four major mass transfer mechanisms to consider:-

- Analyte diffusion through the internal volume of the sample
- Surface desorption of the analyte
- Diffusion of the analyte through the static fluid
- Transport in the bulk supercritical fluid phase

If the rate determining step is intraparticle diffusion, then the rate of extraction will be a function of the particle size of the sample matrix, with a reduction in the particle size leading to an increased extraction rate. Surface desorption of an analyte by a supercritical fluid is an important step in SFE for many sample types. For certain

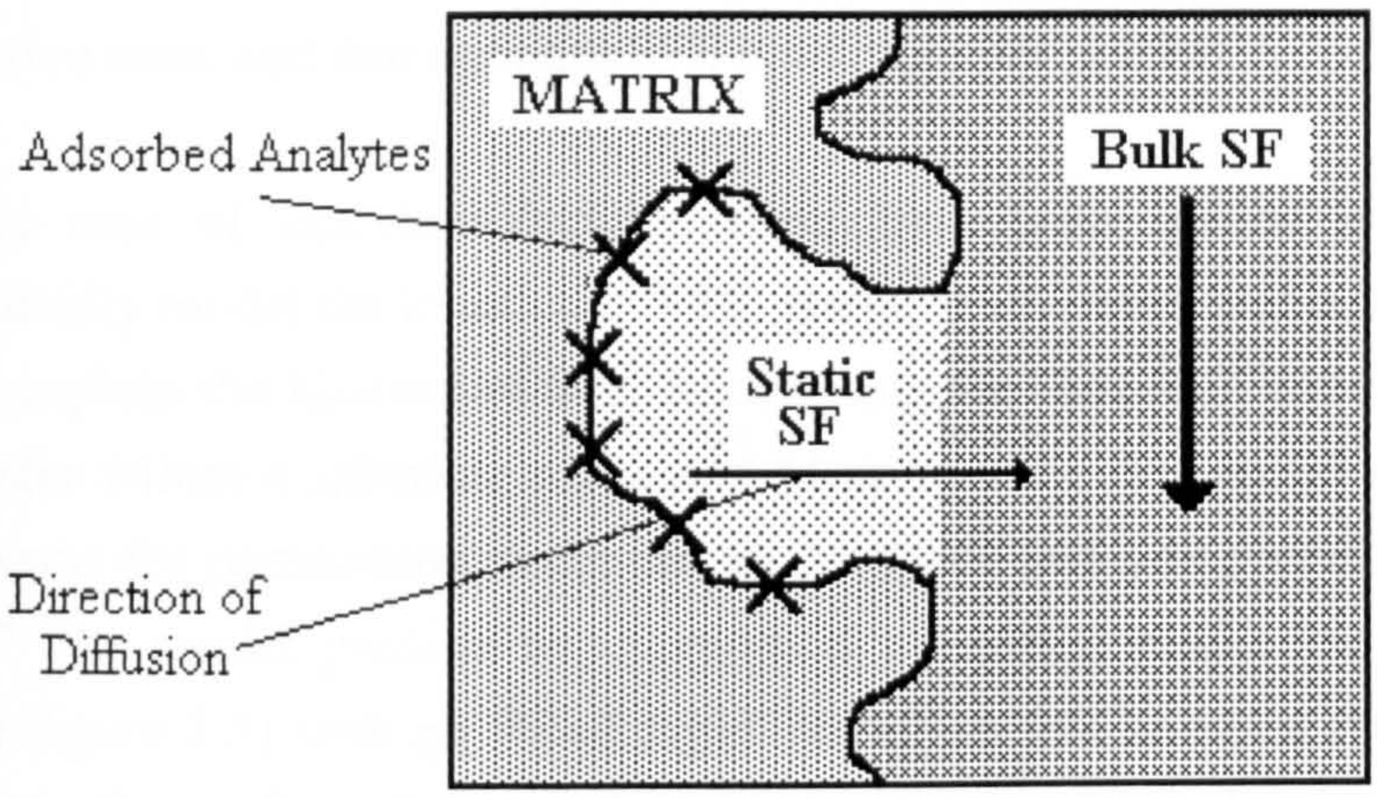


Figure 2.6 Schematic of the SFE of Analytes from Solid Matrices (where SF is supercritical fluid).

analyte-matrix combinations, the "solvent power" of the supercritical fluid alone will not suffice to affect a rapid or complete extraction. The use of a co-solvent such as water or methanol will frequently accelerate the desorption of an analyte from the surface of a sample matrix. Diffusion of the analyte through a surface boundary layer may also kinetically influence analyte extraction. This boundary of static fluid is shown in figure 2.6, with the kinetics of transport through a rate limiting boundary layer primarily depending on the thickness of the layer and the total surface area of the sample matrix. The final stage of extraction depicted in figure 2.6 is the transport of the analyte in the bulk fluid phase, where the analyte is physically swept from the extraction cell.

The effect of extraction flow-rate on the extraction can be used to investigate whether the major limitation to achieving rapid extractions is primarily a "thermodynamic" (distribution of the analyte between the supercritical fluid and the sample matrix at equilibrium) or a "kinetic" (the time required to approach that equilibrium) problem. For samples that show a dramatic increase in extraction rate when the fluid flow-rate is increased, the kinetics of the extraction process appear to be fast, and the extraction will be improved by increasing the proportion of analytes in the extraction fluid by simply exposing the sample to more fluid. Whereas, if there is no large effect of fluid flow-rate on extraction rates, the kinetics of the desorption process or the diffusion through the matrix are slow, and these slow kinetics limit the overall extraction rate. If grinding the solid sample yields faster extraction kinetics, then diffusion was the

limiting factor. However, if grinding the sample does not increase extraction rates, it is likely that the supercritical fluid does not effectively compete with the analytes for matrix active sites, and that stronger SFE conditions are needed.

As in the case of analyte solubility, there have been several methods used to mathematically model the kinetics of SFE. Bartle *et al.* developed a diffusional-based model to explain the kinetics of SFE by adopting the "hot-ball" model that describes heat transfer within a spherical particle.[83] More recently, the model was extended to include terms for partitioning at the sample interface and desorption from the sample surface.[84] The model predicts an exponential extraction profile (similar to the one shown in figure 2.5) with an initial rapid extraction that is associated with analytes located near the surface, followed by a slow diffusion limited period from analytes located in the interior of the matrix. The model was shown to fit experimental data well and was used to extrapolate final concentrations of analytes without the need to fully complete the extraction.

Pawliszyn also proposed a mathematical model to explain the kinetics of extraction in SFE based on equations adopted from engineers and chromatographers.[85] The model assumed that the sample matrix is composed of particles that may be covered by an organic layer which was thought to be a reasonable approximation since many environmental samples contain humic and fulvic material. The native analyte is assumed trapped on the core surface and must undergo several processes before being removed from the extraction vessel. These include (A) mass transport from the matrix to the matrix-fluid interface (*e.g.* desorption from the surface and/or diffusion through the organic layer on the matrix) and (B) partitioning at the matrix-fluid interface. Finally, bulk mass transport in the supercritical fluid (diffusion through the static fluid present in the pore) is also considered. The method reported was used to model both dynamic and static / dynamic SFE techniques, and to investigate analyte-matrix interactions to determine the extraction rate limiting process. More recently the model has been utilized by Langenfeld *et al.* where it was compared with experimentally obtained extraction data.[86] Isotopically labelled spikes were used in experiments where they were simultaneously extracted with native analytes to distinguish between processes A and B and therefore determine the important extraction processes. Although native analytes must undergo processes A and B to be extracted, the isotopically labelled analytes only undergo process B, since they are initially present in the fluid phase. The steps important to SFE were therefore investigated by comparing the extraction time profiles for the spiked and native analytes. A model to describe mass transport was used to predict extraction behaviour, with good

correlation with experimental data. The study showed that increasing the extraction temperature improved SFE kinetics and could be used regardless of whether slow SFE rates were due to poor partitioning in the fluid or limited by strong matrix interactions.

Matrix Effects The least understood step that controls the SFE efficiencies obtained from environmental samples is the partitioning of the pollutant molecules from the active sites in the sample matrix into the supercritical fluid. The large number of possible interactions that might occur between pollutant molecules and an environmental matrix has meant that a fundamental understanding has been impossible to attain.[87] Examples of interactions include the association of pollutants with a variety of inorganic (*e.g.* alumina, silica) and/or organic (*e.g.* humic and fulvic) active sites already discussed in chapter 1. In addition, the extraction of pollutants may be inhibited by physical barriers such as being located in micropores in soil particles or between clay plates, or being coated with a layer of water that may need to be first extracted before the analytes become accessible for extraction. Many of the early SFE investigations were based on the assumption that attaining high solubility in the supercritical fluid should be sufficient to obtain high extraction efficiencies from environmental samples. Unfortunately, this is generally not a sufficient condition to yield high extraction recoveries. In addition to the obvious need for adequate solubility, a successful extraction must overcome the interactions between the analyte and the matrix to affect a favourable partitioning into the supercritical fluid. Often in analytical scale SFE, these matrix interactions are not encountered because "real" samples are not available and laboratory produced fortified samples are used in their place. The spiking of samples with solvent containing the pollutants of interest is useful in determining whether the analyte shows any solubility under the supercritical fluid conditions chosen or in evaluating analyte collection efficiencies but cannot hope to copy the complex interactions between solute and matrix which develop during sample ageing. Burford and co-workers reported up to a 10-fold increase in extraction rates when comparing soil samples spiked with PAHs to samples containing native PAHs.[88] In most cases a 30 minute extraction with pure CO_2 quantitatively recovered (>90 %) the spiked PAHs, but only extracted 25-80 % of the native PAHs. Different methods of spiking (slurry) have been shown to give analytes more realistic interactions with the sample matrix and are preferred over conventional "spot" spiking techniques.[89] However, native samples or commercial reference samples should be used to evaluate SFE efficiency when available.

As well as the effects of pressure and temperature on SFE extraction efficiency, other physical parameters can also have a profound effect on analyte recovery.

Time of Extraction and Static versus Dynamic For obvious reasons, the length of time an extraction is performed is critical to effective SFE. In figure 2.5, it was seen that the amount of analyte extracted shows a non-linear relationship with respect to time. In a typical extraction 50 % is extracted in 10 minutes, but it may be 100 minutes before approximately 99 % is extracted. It is not correct, therefore, to assume that extraction is essentially complete if it has been carried out for two consecutive time periods and the second only produces a fraction of the compound extracted in the first period. In order to estimate the overall time required to complete an extraction, it is necessary to carry out an experimental long extraction and to empirically ascertain the minimum time required to effect quantitative removal of analytes.

However, SFE is further complicated by the way in which the time of extraction is split, as SFE can be performed in two different modes during extraction. The static mode, where once the extraction cell is charged with fluid to the desired pressure it is sealed, is commonly used at the beginning of an extraction, when solubility dictates the extraction rate, to allow the supercritical fluid to "soak" into the matrix. A static extraction period is also essential if modifiers have been added directly to the sample matrix to allow adequate matrix-modifier interaction. After the static extraction is completed, the solvated analytes must by removed from the cell which is performed by a dynamic extraction. In dynamic mode, fresh solvent is continually passed through the cell at a set flow-rate and the analytes are swept from the cell into the collection unit. As with the overall extraction time, the time of each static and dynamic period which make up the complete extraction are difficult to evaluate and are usually empirically chosen. When the analytes are present at a much lower concentration than their solubility in the supercritical fluid, dynamic extraction seemingly offers little advantage over static SFE. However, if the extraction rate is limited by the analyte solubility in the supercritical fluid, dynamic extraction (and high flow-rates) will obviously excel over static SFE.

Flow-Rate and Cell Design Once the kinetic and solubility limitations have been investigated, the effects of the parameters which affect the sweeping of the analytes from the cell can be evaluated. Factors that could potentially control the rate at which an extracted analyte is swept through the sample cell include the flow-rate of the supercritical fluid, cell geometry and volume of the cell (and associated dead volume not occupied by the sample).

The effect of fluid flow-rate can be of great importance in the extraction process and has been mentioned previously in the discussion of kinetic limitations. In general, if

the fluid flow-rate has a large effect on the extraction rate, then it tends to show that the analytes are present in high concentration which therefore saturate the fluid at low flow-rates. Increasing the fluid flow in turn increases the amount of supercritical fluid passing over the sample and thus reduces the effect of ineffective solvation. In contrast, if analytes are present in trace amounts, the flow-rate has little effect on extraction rates. However, in all SFE extraction it is important to ensure that the sample cell volume is continually swept with fresh fluid throughout the dynamic extraction. It has been estimated that the number of cell volumes swept must be a minimum of 4 to ensure efficient removal of analytes[90] as below this there is insufficient contact between the sample and supercritical fluid. The number of cell volumes swept can be calculated for carbon dioxide using equation 2.2.

$$\text{Cell volumes swept} = \frac{\text{Mass of carbon dioxide (g)}}{\text{Density (g ml}^{-1}\text{) x cell volume (ml)}} \qquad (2.2)$$

where mass of carbon dioxide = density of liquid carbon dioxide (0.92 g ml^{-1}) x flow-rate (ml min^{-1}) x extraction time (minutes).

Unfortunately, one consequence of flow-rate not involving the actual extraction is its effect on the collection efficiency of SFE. When a supercritical fluid such as CO_2 is depressurized from supercritical conditions many hundreds of millilitres of gaseous CO_2 passes through the extraction device. If a vial containing a small amount of solvent is used for trapping (probably the most common technique) then the CO_2 bubbling through the solvent can act as an efficient aerosol removing extracted analytes and therefore reducing recovery. An increased flow-rate obviously increases the amount of CO_2 passing through the collection vessel which increases the aerosol effect. Overly high flow-rates should therefore not be used if analyte trapping is a problem.

The extraction cell is normally chosen to minimize dead volume, since this will allow larger samples to be extracted with lower extraction fluid flow-rates. Often the exact cell size needed for various samples is not available. In such cases, the cell may be filled with an inert material (*e.g.* Celite) to reduce the void volume. SFE extraction cells are also available in a variety of shapes as well as sizes. The effect of microextractor cell geometry was investigated on the extraction recoveries of PAHs from C_{18} sorbents.[91] It was found that SFE efficiencies were increased by more than a factor of two by decreasing the cell diameter to length ratio from 1 : 20 to 1 : 1 for the largest PAHs studied. However, it has been reported that when cells are completely

filled with sample, the effect of shape is minimal, when comparing "short-broad" to "long-narrow" cells (with the same internal volume) for the extraction of PAHs from soil.[87]

Sample / Solute Pre-Treatment The physical nature of the sample undergoing SFE can have a significant effect on the efficiency of the extraction and the rate at which it is performed. In general, the smaller the particle size of the sample, the more rapid and complete the extraction will be and sample grinding has been already discussed as a technique for enhancing extraction rates determined by slow diffusion. This effect is largely due to the shorter internal diffusional path lengths over which the extracted solutes must travel to reach the bulk fluid phase.

In addition, the chemical composition of the sample matrix can have either an enhancing or retarding effect on the results that are obtained with SFE. One of the major parameters that influences the extraction efficiency is the presence of moisture in the sample. In general, it is thought that removing any moisture in the sample will allow more rapid extractions to be performed. This can be achieved by freeze-drying the sample or by adding a drying agent to the matrix.[92] The adverse effect of moisture on extraction is due to the fact that highly hydrophilic matrices inhibit contact between the supercritical fluid and the target analytes. Samples containing high levels of moisture can also cause restrictor plugging as a result of the water they contain freezing at restrictor tips and additionally cause problems with subsequent chromatographic detection. However, in some cases, the presence of water may actually aid the recovery of target analytes by acting as an "internal modifier", enhancing the solvating power of non-polar supercritical fluids. An example of this occurs in the extraction of caffeine from coffee beans.[93] As well as the addition of drying agents to samples, other selective additives have been used to enhance analyte recovery in SFE. Examples of additives to affect sample clean-up are discussed in the applications of SFE to the analysis of plant and animal tissues (section 2.3.1.3). For solutes which are strongly bound to their matrix, a strong acid treatment may partly destroy the sample matrix, thereby releasing the analytes. An acid pre-treatment of samples to release dioxins from fly-ash enabled quantitative recovery with pure CO_2, which otherwise required the use of more polar supercritical fluids (N_2O) or a modifier.[94] In fact, the extraction efficiency increased from 9 % to 100 % as a result of the acid treatment.

Supercritical fluid extraction with CO_2 provides a rapid alternative to liquid solvent extraction for the removal of non-polar compounds from a variety of matrices.

However, quantitative extraction of polar analytes calls for addition of organic modifiers to CO_2. *In-situ* chemical derivatization under supercritical conditions is an alternative to modifier addition for increasing the extraction efficiency of polar species. In addition, it conditions analytes for their subsequent chromatographic determination. The functional group that is subjected to derivatization can belong to the analyte or the sample matrix. As a result of derivatization, a less polar substance can be obtained than the original analytes which lend themselves more readily to extraction. On derivatization, the analyte polar groups (hydroxyl, carboxyl) are converted to other, less polar functions (ether, ester) which make the derivative more soluble in supercritical CO_2.

In-situ derivatization-SFE is performed in static mode where the analytes simultaneously undergo derivatization and extraction, after which they are subjected to dynamic SFE. The derivatizing agents most commonly used in SFE are those typically used in GC derivatization. For example, hexamethyldisilane (HMDS) or trimethylchlorosilane (TMCS) have been used to substitute an active hydrogen with a silyl group (tri-substituted silicon atom). This has two major effects on the solute, namely; it decreases its polarity and ability to form intermolecular hydrogen bonds, and increases its thermal stability. Methylating agents have also been successfully used to replace hydrogen with a methyl group and examples of both derivatizing techniques are found in the SFE applications section (herbicides and plant / animal tissues).

2.3.1.2 Instrumentation

Supercritical fluid extraction (SFE) instrumentation is conceptually simple and consists of four basic components, shown schematically in figure 2.7. Many of these components are commonly found in any analytical laboratory as SFE systems contain several individual units which are also found in high performance liquid chromatography (HPLC) apparatus. Consequently much of the early research in analytical scale SFE, before commercial instrumentation became widely available, involved the use of "home-made" equipment.[95,96] The simplicity of the basic apparatus required to perform SFE also has the advantage that "in-house" modification of existing equipment can often be made to suit experimental needs. Many differences in the four basic components do exist and the requirements of a particular extraction may often pre-determine the modifications used. The four different components are discussed in more detail overleaf.

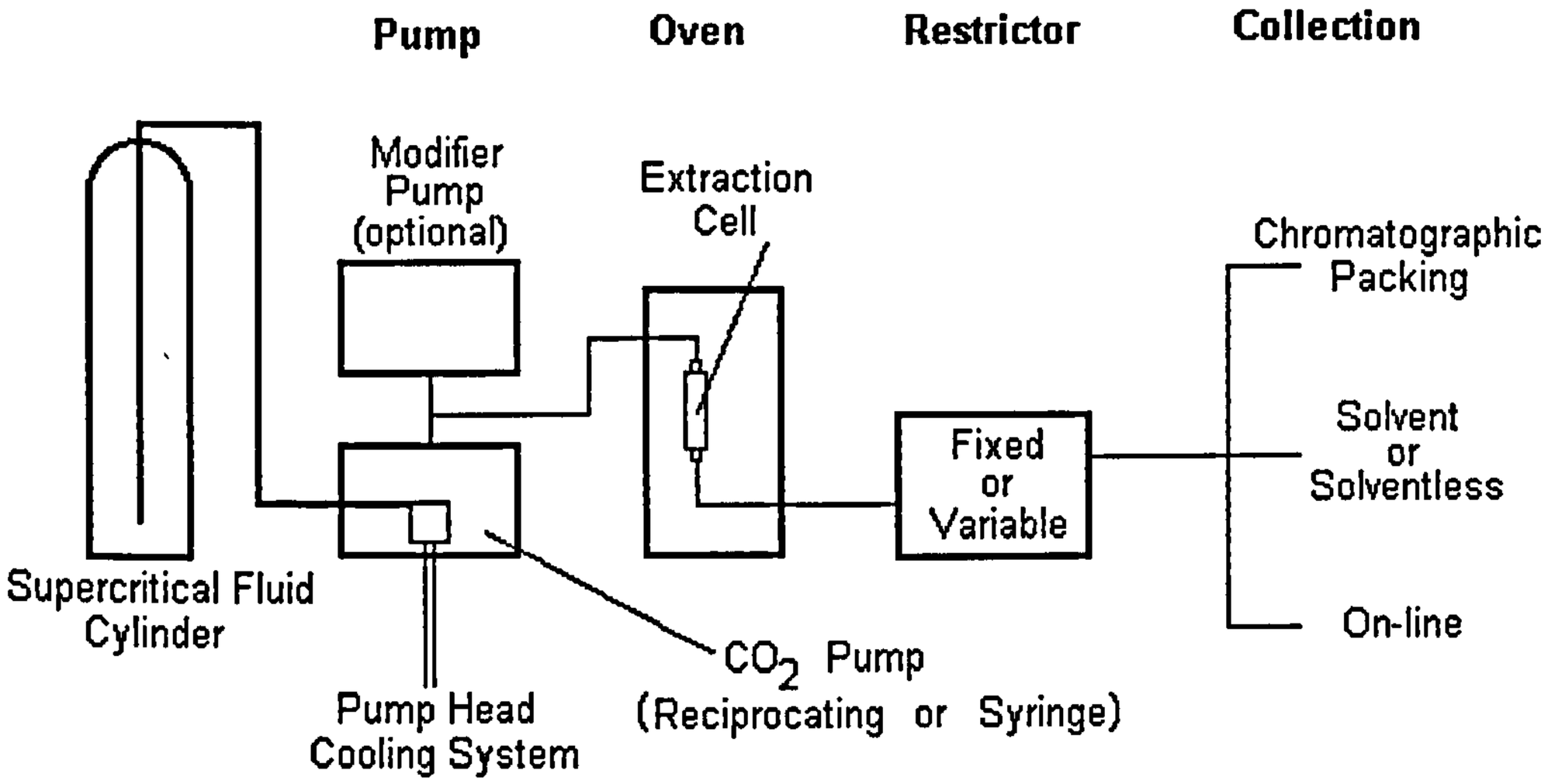


Figure 2.7 Schematic of the Basic Components of a SFE System.

Supercritical Fluids

Many different supercritical fluids have been used to extract analytes from diverse matrices. However, carbon dioxide is the most common and is used in all the SFE apparatus described. Carbon dioxide is contained as a semi-liquefied gas, usually in a steel cylinder and may be purchased in many different purities which in turn have varied costs. High purity supercritical fluids are required for extraction purposes because of the large amount of fluid used in one extraction, where often hundreds of millilitres (liquid) are concentrated into a small amount of solvent.[97] Consequently, even small impurities present in the fluid would cause interferences in the extracted sample. This is particularly true when working at trace levels (ppm levels or below).[98] Carbon dioxide may be purchased as a SFE fluid with a purity of ≥99.9995 % or higher from speciality suppliers such as Air Products or Scott Speciality Gases.

Pumps

The pump in the SFE system is used to deliver a known amount of the supercritical fluid. They are conventionally either a reciprocating type, usually used in HPLC, or syringe type which are often used in supercritical fluid chromatography. The reciprocating pump uses a piston to continually pump the fluid and therefore does not require intermittent refilling with fluid. This is a major advantage over the syringe type of pump which usually holds approximately 150-200 ml of extracting fluid and requires refilling after the fluid reserve has expired. Any pump used in SFE must be able to withstand the high pressures used which may exceed 400 atm. Typical flow-rates used in SFE are between 0.1 and 5 ml min^{-1} and are usually used at constant pressure. A pulse dampner is commonly used in conjunction with a reciprocating

pump to reduce the "pulsing" caused by irregular fluid delivery. Syringe pumps do not suffer from this problem as they can deliver supercritical fluid at a constant rate. Although carbon dioxide is the most commonly used supercritical fluid, other more toxic and corrosive fluids have been utilized including nitrous oxide[99] and chlorofluorocarbons.[100] If fluids such as ammonia are to be used for extraction then any pump must also be able to withstand the corrosive nature of such a fluid.[68]

Most supercritical fluids are gaseous at room temperature, therefore pumps used to deliver the fluids are usually cooled in order to liquefy the fluid and allow efficient pumping. The supercritical fluid is piped from its cylinder in stainless steel tubing which is normally cooled prior to introduction into a cooled pump head. Cooling is conventionally achieved by either a refrigerant bath or by using low-grade carbon dioxide from a second cylinder. Additionally, electronic cooling is also possible by using a peltier cooling system.

A second pump is often incorporated into a SFE system to allow the continuous addition of a modifier to the sample. A normal HPLC solvent delivery pump may be used for this task without modification. Modifier can also be added directly to the sample, prior to extraction, or by using pre-mixed cylinders containing the required percentage of modifier. However, when modifier is added directly to the sample, a long static extraction period is required to allow sufficient modifier-sample interaction, which extends the overall extraction time. Additionally, when the dynamic extraction period is begun, all of the modifier is quickly swept from the sample and therefore the increased polarity benefits of the modifier are lost for the majority of the dynamic extraction. Pre-mixed cylinders have several disadvantages, which include the high cost when compared to normal fluids and their inflexibility. Also the pre-mixed cylinders tend not to deliver uniform amounts of modifier as their contents are gradually exhausted.[101] These disadvantages may mean that a second pump, despite the additional cost, is the most convenient way in which to add percent amount of modifier when required.

Oven

An oven is used in SFE to maintain the temperature of the fluid above its critical temperature when in contact with the sample. The sample of interest is placed in an extraction "cell" which in turn is housed inside the SFE oven. Again, the ovens used are in common with those used in HPLC, or GC if high temperature extraction is to be performed.[102] The oven must be capable of allowing small increases in temperature and be large enough to hold the extraction cell, together with valves and tubing. In a

conventional system, the pumped supercritical fluid is carried into the oven compartment by stainless steel tubing (the modifier, if any, will also be present and mixed with the fluid) and is pre-heated to the required extraction temperature before entering the cell containing the sample. This may be achieved by coiling the tubing many times inside the oven before it is connected to the cell.

Extraction cells can be purchased or constructed from conventional tube fittings, but care must be taken to ensure all of the extraction system components are rated for the working pressures. Cells are usually made from stainless steel although PTFE extraction cells are used with some commercial instrumentation.[103] Cells can differ in size and geometry and are dependent upon the sample of interest. In general, it is common practice not to use cells which are overly large in comparison to the sample to be extracted. Extraction cells which are used to extract solid samples containing little water often resemble empty HPLC columns and indeed empty columns are frequently used for this purpose. Small porous frits are connected to each end of the cell, which allow only the passage of extracting fluid and prevent any of the sample escaping. Other designs are used, where the supercritical fluid is removed from the headspace at the top of the cell, when samples may contain large amounts of water which would be simply flushed out with the former design.

Restrictor

A restrictor is required to constrict the flow of supercritical fluid and maintain a back-pressure in the system above the critical pressure of the extracting fluid. Two types of restrictor are used, fixed and variable. Fixed restrictors are by far the most common as they simply consist of some form of capillary constructed from fused silica or stainless steel tubing. The dimensions of the fixed restrictor are empirically selected to give the desirable fluid flow velocity at the required pressure and temperature settings.[104] Many different fixed restrictor designs have been utilized but all suffer from the problem of plugging when large amounts of analyte or matrix are extracted by the supercritical fluid.[105] This problem can be reduced by externally heating the restrictor with a heating block or gun.[106,107] This also helps to reduce the amount of frozen carbon dioxide formed at the end of the restrictor by the Joule-Thompson effect.[95] In addition to the problem of plugging, the fixed restrictor has another associated difficulty in that when the pressure of the supercritical fluid is increased, the fluid velocity also increases. Therefore it is not possible to keep the fluid pressure and flow-rate constant at the same time without changing restrictors. If the flow-rate becomes too high, analyte may be lost from the collection system due to the aerosol caused by the depressurized extracting fluid containing the analyte. Although fixed

restrictors do suffer from the inherent problem of plugging and increased flow-rate with pressure, they are inexpensive and easily replaced and therefore are often the preferred choice over variable restrictors.

Most modern variable restrictors are based on a high speed pulsing regulating valve which can be electronically controlled. This device is capable of controlling the cell outlet pressure independently of the mass flow-rate of the fluid. The restrictor controls the flow at the end of the SFE system and is therefore called a "back-pressure regulator" (BPR). The fluid flow-rate is then set at the pump and maintained at a constant rate by the BPR which is programmed with the desired extraction pressure. Total computer control of the entire SFE system is possible using some commercial instrumentation and allows the analyst to set all extraction parameters from the one console.[90] Variable restrictors of this type are not easily blocked by extracted analyte or sample matrix and are supplied with a thermostatted heating block to reduce the likelihood of analyte loss due to deposition in the BPR. Variable restrictors are obviously much more expensive and require regular maintenance and part replacement which is the main reason for their slow acceptance when compared to the fixed type.

Analyte Collection

Once the supercritical fluid, containing the extracted analytes has passed through the restrictor, depressurization occurs. The fluid is no longer supercritical and the analytes simply "drop-out" of solution with the gaseous fluid allowed to escape. A means of collecting the analytes after this stage is therefore required and the system used is dependent upon whether the extraction is be performed in off-line or on-line mode. Typical collection devices, for off-line SFE, consist of either a closed vessel which is empty or contains a small amount of an appropriate solvent [108] or a trap incorporating some form of solid packing material.[109] The packing material used is dependent on the nature of the analytes to be collected and is often based on a chromatographic stationary phase. Once the analytes have been deposited from the fluid onto the packing material, the adsorbent is back-flushed with a small volume of rinse solvent. The adsorbent material chosen together with the rinse solvent require careful consideration if good recoveries are to be obtained.[110] One problem associated with off-line collection using the first method is the aerosol caused by depressurized extracting fluid which can remove analyte from the collection unit during extraction.[111] The main difficulty encountered when using a solid packing material as an analyte trap is when modifiers are to be used, which may flush quantities of the adsorbed analyte from the trap during extraction.

The final method of collection is to directly couple the SFE to a chromatographic system in an on-line extraction.[112] Supercritical fluid chromatography[113] or gas chromatography[114] are the preferred modes of detection. The way in which the SFE is coupled to the chromatograph is dependent upon the injection system used. The three most common ways of coupling are:[115] 1. to use an external sample loop through which the SFE extract flows. The extract is analyzed by rotating the sample loop valve so that a representative sample of the extract is introduced into the chromatograph; 2. to utilize an external trap to recover analytes from the depressurized supercritical fluid. Trapping of the analytes is achieved using sorbent traps or a cryogenic loop. After the extraction is completed, the analytes can be introduced into the GC by sweeping the trap with carrier gas; and 3. frequently the restrictor is simply placed directly into the injection port with the analytes focused at the front of a cooled column. The advantages of on-line analysis in SFE are similar to other on-line detection systems and include, increased sensitivity (since all of the extracted analytes are focused at the front of the analytical column), a reduction in the amount of sample handling required and therefore, a reduced risk of contamination.

2.3.1.3 Environmental Applications

Supercritical fluid extraction has become an important technique in analytical sample preparation, as is apparent from the number of publications dedicated to the subject in recent years. In particular, the field of environmental analysis has been focused upon as the area where the majority of SFE research is undertaken. This is probably due to the vast number of soil samples and related matrices (sediments, dust and sludge) which require routine analysis in environmental laboratories and therefore consume a large amount of time in their sample preparation using conventional solvent extraction. These solid samples are directly amenable to conventional extraction with supercritical fluids and in addition, many of the analytes of environmental interest (PAHs, PCBs and many pesticides) are non-polar and therefore compatible with extraction using supercritical carbon dioxide. Other solid matrices of environmental concern have been successfully extracted using SFE, including plant and animal tissues. However, SFE sample preparation of aqueous samples has yet to become widely accepted because of difficulty in retaining the sample in conventional SFE apparatus.

SFE from Soil and Related Samples

Chlorinated Organic Compounds

Many different classes of pollutants have been extracted from environmental solids using supercritical fluids, although certain groups are more commonly studied. A vast amount of work has been carried out on the extraction of halogenated organics from soils including organochlorine pesticides (OCPs), PCBs, and dioxins. This may be primarily due to their great environmental concern based on their toxicity to mammals and, as mentioned previously, their non-polar nature giving compatibility with carbon dioxide as the extracting fluid. The majority of samples studied in SFE are laboratory-prepared spiked samples as contaminated native soils are often difficult to obtain. Lohleit *et al.*[116] showed quantitative extraction of spiked OCPs and PCBs in soil at low ppb levels with the extracts being trapped in a specially designed collection unit. A study on many different OCPs and organophosphorus pesticides (OPPs) was undertaken by Lopez-Avila *et al.*[117] who spiked sand with the pesticides and extracted under various conditions. A simplified factorial design was used to optimize seven extraction variables which allowed the main effects to be estimated. Overall it was concluded that recovery was most affected by extraction time and pressure and least affected by modifier volume. However, the ineffectiveness of artificially fortified solid samples to mimic the behaviour of real samples was illustrated by a study showing excellent recovery of PCBs from spiked sediment (between 87 % and 106 % at high density, depending on the congener) but low recovery from a certified sewage sludge sample (between 7 % and 52 % at the highest density studied).[118] After optimization, the recoveries obtained by SFE ranged from 67 % to 134 % depending on the PCB congener.

The effect that altering extraction parameters has on extraction efficiency has been studied by many workers. The dependence of extraction of PCBs on sorbent (matrix) type and extraction cell dimensions was investigated by Furton and Lin.[119] It was shown that the sorbent type greatly affected the recoveries of all PCBs studied. Further work was reported in this area by the same authors with reverse phase chromatography sorbents binding PCBs strongly.[120] Cell dimensions did affect recoveries from these sorbent matrices (and also from phenyl sorbents) but showed no difference with other materials. This was postulated to be due to a chromatographic retention mechanism taking place during the SFE stage. The two main variables in SFE, pressure and temperature, which dictate the density of the extracting fluid, were examined for their effect on the extraction efficiencies of PCBs (and PAHs) from urban air particulates and river sediment, respectively.[121] A marked increase in

recovery was observed for both samples when the temperature was increased from 50 to 200 °C even at low extraction pressure. This indicates that desorption was the SFE rate limiting step and that increasing the temperature may be an alternative to the addition of modifier to extraction analytes which are tightly bound to their matrix. The same matrices were also studied by Schantz and Chesler[122] where the analytes were extracted at 345 bar and 40 °C for four hours. SFE compared well with Soxhlet for extraction of PCBs in sediment.

Many of the studies in environmental solid samples compare the performance of SFE with more conventional liquid extraction techniques. Richards and Campbell[123] compared SFE with Soxhlet and sonication methods for the extraction of chlorobenzenes from spiked soil samples. It was found that SFE gave the highest extraction efficiencies of all three methods using 2 % methanol modifier in carbon dioxide after only 30-40 minutes. Snyder *et al.*[124] also compared the three techniques for the extraction of twelve OCP (and OPPs) from four different soils. SFE was found to give comparable recovery with the highest precision for spiked soils and performed equally as well as the sonication method for extraction of pesticides from native top soil. A comparison of SFE with Soxhlet extraction and normal solvent extraction when extracting OCPs and PCBs from both a low and high organic content soil was also reported.[125] The more volatile pesticides showed poor recovery and this was associated with a collection problem. When the collection solvent was changed from hexane to iso-octane recoveries of the more volatile analytes showed a significant increase. All three techniques gave good recoveries from the sandy soil. However, solvent extraction was incapable of quantitatively extracting from the peat soil. SFE was found to give a far cleaner extraction than the other two methods which was advantageous in the subsequent detection.

Sulphur is often present in contaminated soils and if co-extracted with the analytes of interest, usually interferes with chromatographic analysis. In addition, soils containing percent levels of sulphur also pose a different problem when SFE is used as the sample preparation technique, since the sulphur can be co-extracted and quickly block the restrictor. A recent publication successfully utilized SFE to extract PCBs from sediments containing approximately 1.5 % sulphur by use of an *in-situ* clean-up.[126] The process involved adding copper and sodium sulphate to the sediment portion (2 g) inside the extraction cell. Supercritical fluid extractions were performed initially in static extraction mode (20 minutes) with pure CO_2 at a density of 0.75 g ml^{-1} at 60 °C followed by a 40 minute dynamic extraction at the same density and temperature and at a flow-rate of 1 ml min^{-1}. Complete analysis was achieved in under 2.5 hours

including the GC analysis time. The final analytical method was compared with Soxhlet extraction, applied to a certified sewage sludge sample, and gave quantitative recovery and detection limits of 1-2 ng g^{-1} dry weight and standard deviations of less than 10 %.

Other examples of the SFE of chlorinated organic compounds include the work by Burk and co-workers who used a home-made SFE system to quantitatively extract PCBs from soil and sand.[127] However when phenoxy acid herbicides (more polar analytes) were extracted, the highest recovery obtained was six percent. This was not due to limiting solubility in carbon dioxide but to the matrix tightly binding the polar herbicides. Other extraction fluids were investigated and N_2O was found to give more extensive and rapid extractions than all the other fluids used. A preliminary report, produced by Isco Inc.[128] investigated the extraction of PCBs (and diesel fuel) from a reference sediment with supercritical carbon dioxide. Various extraction conditions were studied including the effect of using wet or pre-dried sediment. It was found that even with the use of a drying agent, PCBs were not extracted from wet sediment. The addition of an organic modifier (5 % methanol) and a fluid density of 0.836 g ml^{-1} were required to quantitatively extract PCBs from the dry reference sediment. Brady and co-workers[129] reported on the use of supercritical carbon dioxide to extract PCBs, DDT, and toxaphene from contaminated soils. The two major factors which influenced extraction efficiency were found to be the soil's water and organic content with incomplete removal of DDT and toxaphene possible from high organic content topsoil. The presence of water slowed all extractions although the final amount extracted did not differ from that extracted from dry soils.

SFE has often been directly coupled to a chromatographic detection system as this offers some advantages over off-line SFE. In on-line SFE the entire extracted sample is introduced into the system therefore greater detection sensitivity can be obtained. There is also less chance of sample contamination because there is no intermediate handling of the extracted analytes. Initial studies have focused on coupling SFE to SFC since carbon dioxide is used as both the extraction fluid and the chromatographic mobile phase, although much research into coupling SFE to GC has been carried out. Andersen and co-workers discussed some theoretical considerations involved in using SFE as a method for sample introduction in chromatography.[130] However, the most common technique used in on-line SFE of environmental samples is coupled SFE-GC.[115] The various methods in which SFE is commonly coupled to GC have already been discussed in section 2.3.1.2. Examples of their use include work by Raymer and Velez[131] who incorporated a Tenax-GC (a polymer of 2,6-diphenyl-p-phenylene

oxide) adsorption step prior to GC for on-line SFE-GC extraction of a range of pesticides and PCBs from sea sand. The addition of the Tenax trap gave better peak shapes than direct SFE-GC because of the refocusing effect. It also allowed the methanol modifier, used in the extraction, to be removed before flushing the analytes onto the column which previously caused a "hump" in the baseline in conventional SFE-GC. The same technique was also used to extract PCBs directly from a Tenax adsorbent trap[132] which utilized a reusable restrictor, and also from a certified sediment which gave excellent agreement when compared to conventional extraction methods.[133]

One other class of chlorinated organic compounds which have been extensively studied in the field of SFE are the polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). Dioxins and furans are produced by the burning of chlorinated chemicals and therefore much of the research into their analysis has focused on their extraction from fly ash produced by incinerators. Both PCDDs and PCDFs were extracted from fly ash using SFE with both CO_2 and N_2O.[94] Pure CO_2 was found to give almost no extraction even though dioxins are readily soluble at increased pressures. This was believed to be due to strong matrix adsorption of the dioxins. Modified CO_2 extractions were then tried with methanol and benzene. Methanol did not to improve the recovery although benzene yielded almost 100 % recovery. A move to replace benzene with less toxic toluene resulted in lower recoveries. A different approach, where the matrix is destroyed by exposure to a strong acid, was used. Extraction with pure CO_2 only, now gave quantitative recoveries comparing well with a conventional 20 hour Soxhlet extraction using benzene. Finally N_2O was used as an extracting fluid and gave recoveries >74 % for all dioxins studied without the need for modifiers or acid pre-treatment. A similar study was conducted by Onuska and Terry[134] who extracted pre-treated fly ash (3 % hydrochloric acid) and analysed for dioxins using high resolution GC-MS. Fourteen separate experiments using both CO_2 and N_2O, modified with methanol and toluene concluded that the addition of formic or hydrochloric acid to the sample greatly increased the recovery of dioxins. The optimum conditions for extraction were found to be N_2O + 5 % methanol at 400 atm and 45 °C after pre-treatment with acid. The results were once again comparable with a Soxhlet extraction. Municipal incinerator fly ash was also extracted for dioxins in a recent paper with N_2O as the extracting fluid.[135] The analytes were analysed by GC-ion trap mass spectrometry giving the low detection limits required. Both Florisil and CO_2 were used as methods of clean-up to remove interfering compounds from the sample prior to analysis. Sample clean-up

with Florisil was found to give both a cleaner extract and higher recoveries than the CO_2 clean-up procedure.

Dioxins have also been extracted from a soil matrix[136] using pure CO_2 at 300 atm and 40 °C. Although high levels of dioxins were obtained from spiked samples, indicating the adequate solubility of dioxins in CO_2, native soil known to contain levels of dioxins was found to give approximately 30-50 % of the levels observed using Soxhlet techniques. This illustrates the problems related to SFE of native samples which bind analytes on active sites. A later publication used chloro-organic compounds other than dioxins to initially optimize SFE operating parameters using a factorial design.[137] The final optimum conditions used included a 5 minute CO_2 extraction (with 200 µl methanol added) at 0.25 g ml^{-1} density, followed by a second sequential extraction at 0.8 g ml^{-1}. Both extractions were performed at 80 °C. When dioxins were substituted for the chloro-organic compounds, the second extraction stage was lengthened to 20 minutes to account for the lower volatility of the dioxins. The optimum conditions were compared with Soxhlet extraction and in all cases SFE gave improved recoveries. In addition, because dioxins are hydrophobic and fat soluble, they can accumulate in fat-rich tissues. SFE has been used to remove dioxins from cod liver oil samples containing different concentrations of fatty acids.[138] The efficiency of the extraction was found to be dependent on the applied pressure and on other conditions, such as fatty acid content and entrainer added to the carbon dioxide. In general, over the range of dioxins studied, the optimum supercritical conditions were found to be an extraction pressure of 150 bar at 40 °C with no entrainer added. Under these conditions, approximately 25 % of dioxins were found in the first 10 % of the oil extracted.

Organophosphorus Pesticides

Another class of pollutant which has been frequently extracted by SFE are the organophosphorus pesticides, or OPPs. A wide range of OPPs with different polarities was extracted from soil.[139] Initial studies used an inert matrix (glass wool) to confirm that the pesticides were being trapped efficiently in an ethyl acetate collection solvent. The OPPs were then spiked onto soil samples using both a spot spike and a slurry spiking method. Recoveries from the slurry spiked soil were vastly reduced compared to the spot spiked sample indicating that slurry spiking is a more representative way in which to prepare laboratory samples as it gives an indication of solute-matrix interactions. Various modifiers were added to the sample to improve extraction of the slurry spiked OPPs with methanol giving the greatest recovery. Modified carbon

dioxide extractions were comparable with solvent extractions with ethyl acetate in a fraction of the time.

An extraction scheme for the analysis of 4-nitrophenol and an OPP derivative of nitrophenol (parathion) from soil using a novel detection system has been developed.[140] Enzyme-linked immunosorbent assay (ELISA) was used for direct analysis of 4-nitrophenol and for parathion after oxidation to paraoxon and hydrolysis. ELISA has certain advantages over gas chromatography which include greater sensitivity, wider dynamic range and faster analysis. It is also directly compatible with SFE if a water based collection solvent is used and can analyse extracts with no further sample preparation. Extraction was once again comparable with solvent extraction using ethyl acetate, but the greater sample throughput allowed rapid screening of environmental samples.

Top soil, clay, and river sediment spiked with OPPs (and OCPs) were extracted under different density and temperature combinations.[141] Extraction recovery increased with an initial steep rise when fluid density was increased. Temperature had little effect on extraction of both classes of pesticide but dichlorvos, the most volatile of the pesticides studied, did show a significant decrease in recovery when the temperature was increased above 60 °C. The moisture content of the soil was found to increase the recovery of the more polar pesticides by acting as a modifier, although as the amount of water added was increased above 5 % the recovery was reduced.

Herbicides

In recent years, herbicides have generally become the most widely used class of pesticide and therefore have generated much interest in alternative analysis techniques which do not require the use of organic solvents. Certain classes of herbicide have been focused on in SFE applications literature, with perhaps the most work being carried out on the triazines. Janda *et al.*[142] extracted s-triazine herbicides from spiked sediment using carbon dioxide at 230 bar and 48 °C. All herbicides studied, with the exception of simazine, were extracted with recoveries above 90 %. Simazine required the use of a methanol entrainer added directly to the sample to increase the extraction efficiency. The poor recovery was linked to its low solubility in low-polarity solvents. In a similar study, s-triazines and phenylurea herbicides were extracted from spiked sediment, only after the supercritical CO_2 used was modified with acetone.[143] SFE was found to be much faster than Soxhlet and thermally labile phenylureas proved to be less susceptible to thermal degradation using the SFE technique. In an evaluation of the extraction efficiency of SFE for removal of atrazine from an un-spiked soil

containing only field residues, near quantitative recovery was obtained by a combination of high CO_2 density at moderate temperature and by using 5 % methanol added to the CO_2 in the dynamic mode.[144] However, no atrazine degradation products were included in the study. The potential for SFE to isolate polar triazine herbicide congeners, including degradation products, in soil has been demonstrated.[145] In this study, the use of SFE was evaluated as an alternative to the classical aqueous methanol or dilute aqueous acid as extraction methods for the removal of atrazine, cyanazine and two atrazine metabolites (desethylatrazine and desisopropylatrazine) from soil. Several combinations of co-solvents, added both to air-dried soil and to the carbon dioxide extracting fluid, were evaluated. In a more recent publication by the same authors, the identical analytes were removed from several different agricultural soils using SFE.[146] Recoveries for each analyte (fortified at the 0.1 to 2.0 mg kg^{-1} range) varied between 25 and 120 % depending upon the soil matrix and the analyte extracted. A single extraction, using co-solvent modified (methanol) CO_2 was sufficient for the extraction of all analytes. The addition of both water and methanol to the air-dried soil prior to CO_2 flow enhanced the recovery of all analytes. Additionally, HPLC analysis indicated fewer co-extracted interferences present with the SFE method when compared to extractions using pure liquid solvents.

Other commonly studied herbicides within the field of SFE include the urea herbicides. Fahmy *et al.*[69] reported the effect of a modifier on extraction of urea herbicides from soil and clay. Mechanisms on modifier interactions between both the solute and matrix are proposed with the extent of matrix swelling, due to the modifier, observed in a high pressure view cell. A direct correlation between diuron extraction from montmorillonite clay and percentage swelling of the matrix was observed at different pressures and constant temperature. It was deduced that an equal mix of water and methanol modifier increased recovery of tribenuron methyl from plant tissue. This was postulated to be due to the water acting as a matrix swelling agent whilst the methanol acted as the analyte solubilizing agent. Extraction and analysis of urea herbicides has also been achieved in an on-line SFE configuration. A SFE-SFC protocol was used to extract and analyze the herbicides linuron and diuron from a sandy loam soil and wheat.[147] After initial SFE optimization, carried out in off-line mode, the two techniques were coupled with flame ionization used as detection. A modifier (methanol or ethanol) was required to achieve quantitative extraction which eluted as a solvent front on the chromatogram. Both capillary and microbore columns were investigated with better sensitivity being obtained with a microbore column and larger sample loops.

Another family of herbicides, sulfonylureas, have also been successfully extracted using SFE techniques. Chlorosulfuron and metsulfuron methyl were extracted from four different soils using supercritical carbon dioxide combined with methanol and water as modifiers.[148] Before commencement of the extraction, 80 µl of methanol and 200 µl of water were added as modifiers, and the cell vigorously shaken. The extraction temperature was kept constant at 50 °C with the pressure of the carbon dioxide at 370 bar. After a 10 minute static extraction, the flow was set at 4 ml min^{-1} and the sample extracted for 8 minutes. The recovery reported was good (>80 %) for all types of soil studied except for those with high organic content (*ca.* 50 %). Metsulfuron methyl and its metabolites have also been studied in on-line mode using SFE-SFC from soil, plant materials and a cell culture medium.[149] The system utilized a switching valve with a loop attached to effect the transfer of extracted analytes to the chromatograph. Enhanced recoveries were obtained at CO_2 flow-rates between 2 and 5 ml min^{-1} with the extracted components collected if further analysis was required. An alternative to carbon dioxide was evaluated for its capability to extract sulfonylurea herbicides (chlorosulfuron and metsulfuron methyl). Trifluoromethane (CHF_3) was used because of its large dipole moment (1.6 D) and easily accessible critical parameters (5.0 MPa and 26.3 °C), making it an excellent candidate for extracting polar compounds.[150] In comparison with other commonly used supercritical fluids such as CO_2, methanol-modified CO_2, and chlorodifluoromethane ($CHClF_2$), CHF_3 showed a 30 % increase in extraction efficiency of the sulfonylurea herbicides over pure CO_2. However, 2 % methanol-modified CO_2 proved the best fluid and allowed >90 % recovery. Results with $CHClF_2$ matched those obtained by Soxhlet extraction. CHF_3 has also been compared with CO_2 for the extraction of a range of pesticides and herbicides (including diazinon, malathion, atrazine, and desethylatrazine) from silanalized glass beads.[151] A 15 % increase in extraction efficiency was obtained when using CHF_3 over CO_2, although the recoveries are not quantitative (maximum of 91.8 % under all conditions) and no selectivity of extraction was reported.

Other, more polar classes of herbicides have been extracted using supercritical fluids, although carbon dioxide has proved ineffective in their removal without sample treatment. Chlorophenoxy acid herbicides (including 2,4-dichlorophenoxyacetic acid, 2,4-D) were extracted from soil samples with CO_2 after *in-situ* analyte derivatization.[152] The derivatizing agents were used to methylate the analytes, converting them to their corresponding methyl esters, and therefore reducing their polarity allowing efficient extraction using CO_2 only. Tetrabutylammonium hydroxide combined with methyl iodide proved to be the most efficient methylating

agents although several others were tried (trimethylphenylammonium hydroxide, benzyltrimethylammonium chloride, and benzyltriethylammonium chloride). The extraction was carried out at 400 atm and at a temperature of 80 °C for 15 minutes static, followed by 15 minutes dynamic at approximately 1.5 ml min^{-1}. Recoveries obtained ranged from 57.4 % to 141.0 % depending upon the analyte and the soil type. Supercritical carbon dioxide was also demonstrated to be a clean and rapid alternative to conventional solvent extraction procedures for the removal of 2,4-D from soil samples.[153] A comparison between various sample pre-treatments was undertaken and included, silylation (with hexamethyldisilane and trimethylchlorosilane), methyl esterification (using BF_3 (14 %) / methanol solution), ion-pairing (utilizing a 0.2 *N* methanolic solution of m-trifluoromethylphenyl trimethylammonium hydroxide), and finally, ionic displacement (involving the addition of 0.2 ml of an aqueous solution containing 1 *mM* phosphate as phosphoric acid). Methyl esterification and ionic displacement during SFE proved the most promising approaches for quantitative recovery. Although the SFE procedures were not optimized, comparison between SFE and a standard Soxhlet extraction method demonstrated the potential for improving the sample preparation of highly polar substances using SFE-CO_2 extraction.

Polyaromatic Hydrocarbons

Probably the most reported environmental application of SFE is in the sample preparation of solid samples containing polycyclic aromatic hydrocarbons (PAHs). Their great interest in the field of SFE is partly due to their ubiquitous presence in the environment which has led to their constant monitoring in soils and related samples in the analytical laboratory. The standard sample preparative technique prior to their analysis is the time-consuming Soxhlet extraction. Its high usage of organic solvents combined with a lengthy extraction procedure has fuelled the demand for faster extraction techniques to be developed. In addition, PAHs are often used as test analytes in SFE as they represent a wide range of molecular masses and boiling points. They are also relatively non-polar and should therefore be directly amenable to SFE with supercritical carbon dioxide.

Several examples illustrate the successful use of pure CO_2 to extract PAHs from environmental solids. PAHs were extracted from pre-ground coal, by both static and dynamic modes of SFE with the PAH extracts being analyzed by GC-MS showing 15 classes of PAH present in the coal sample.[154] Dynamic extraction was found to give both a faster extraction and a greater yield of extracted material. However, no mention of quantitative recovery was made. In many extractions from contaminated soil, SFE

may still have to be followed by further sample clean-up before the extracts can be analysed. The clean-up is required to remove co-extracting organic compounds which will interfere with subsequent detection.[155,156] Columns commonly containing silica gel are used for this purpose. However, other column types can be used (Sephadex LH20). Although the sample throughput time is obviously lengthened, this increase in preparation time is more than compensated for by the cleaner analysis obtained. A novel collection system was investigated which utilizes a no flow restrictor for the analysis of PAHs and *n*-alkanes (also PCBs) from gasoline and diesel fuel.[157] Conventional problems associated with linear restrictor plugging are eliminated as the static extraction is simply depressurized through a stainless steel tube into an empty collection vessel. Quantitative recovery of all *n*-alkanes greater than C_{10}, all PCBs and most PAHs was obtained indicating that the rapid depressurization of carbon dioxide did not displace analytes. The collection of the more volatile analytes showed a dependence on the extraction temperature as this determined the collection vial temperature. The higher molecular weight PAHs were not exhaustively extracted from the matrix and required a larger volume of carbon dioxide. In addition, pure CO_2 has been utilized in a field-portable SFE unit for the rapid characterization of contaminated soils.[158] The SFE results reported compare well with laboratory based Soxhlet extractions on the same samples, although the concentration of PAHs removed is lower in all cases.

The examples above show the ability of pure CO_2 to extract non-polar PAHs from environmental solids. However, the publications cited either do not comment on the quantitative aspect of the extractions or show results which are poorer than those obtained by conventional solvent extraction. The poor performance of pure supercritical CO_2 to extract PAHs is not due to a lack of solubility in the supercritical fluid but to strong binding of the solid matrix towards the analytes of interest. The ability of SFE to quantitatively extract analytes can be enhanced by the addition of modifiers to the CO_2 and the following references investigate the use of various modifiers to quantitatively remove PAHs from solid samples.

The most common modifier used to improve the extraction efficiency of CO_2 is methanol. Coal tar contaminated soil, containing over 0.1 % PAHs was treated by SFE with a CO_2 / methanol modified extracting fluid to remove PAHs and other pollutants.[159] After extraction for seven hours, 92 % of the pollutants were removed as was shown by extensive analysis by GC-MS. This resulted in a virtual elimination of toxicity measured by two short term assays shown as a function of bioluminescence decrease in *photobacterium phosphoreum*. Methanol was also used as a CO_2 additive

to improve the extraction of PAHs from a marine sediment.[160] However, the best results were obtained when using toluene-modified CO_2 as a solvent at 400 atm and 140 °C. Toluene is often used as the preferred extraction solvent to remove PAHs from solids because of its similar aromatic structure. A similar study again used methanol (5 %) or toluene (10 %) to enhance the extraction efficiency of CO_2.[161] Spiked samples were used initially to optimize extraction parameters. Recoveries between the range of 88 % and 101 % were obtained for all 15 PAHs studied at pressures of 40 MPa and temperatures of 80 °C. When real samples were extracted, drying agents were used to trap residual water and elemental copper granules were used to trap organosulphur compounds. The use of copper as a scavenger for sulphur in PAH contaminated soils has previously been demonstrated.[162] A certified marine sediment sample containing a high concentration of sulphur but low levels of PAHs was extracted from the cell and the eluants passed through a copper scavenger column containing 2 g of granular copper and finally through a conventional fused silica restrictor. This was found to effectively remove the elemental sulphur without changing PAH recovery.

Other common organic solvents have been used as supercritical carbon dioxide modifiers in the extraction of PAHs. Dichloromethane (DCM) was used as a static modifier to extract PAHs from polluted soil samples as an alternative to conventional methanol modified CO_2 extraction.[163] The added DCM has the power to penetrate the soil particles and render the soil aggregates soluble therefore increasing contact between the soil particles and extractant. Recoveries of almost 100 % were obtained using the DCM modified CO_2 with the chromatograms obtained being fully comparable to those from organic solvent extraction. Both DCM, methanol, and water have been used as modifiers after pure CO_2 was found incapable of quantitatively extracting PAHs from sediments.[164] The modifiers were either added directly to the sample prior to commencement of extraction or by use of a second pump. With the direct spiking method, a ratio of 1:1:1 (750 µl in total) of the three modifiers was found to give the highest recovery, although no reason for this was given. However, when a second pump was used to deliver a 1:4 mixture of methanol / DCM (the sediment was wetted with water to a 50 % moisture content before extraction), the same results were obtained in less than half the time of the initial experiments. In a recent publication, the presence of a modifier was proven essential for quantitative extraction of PAHs from a native sample, although unnecessary in spiked samples.[165] Several different common solvents were investigated as modifiers (hexane, DCM, acetone, water, and methanol) with a dramatic improvement in extraction efficiency observed with water or methanol (50-250 % and 75-300 %, respectively) compared to

those obtained without modifier addition. Overall, for all PAHs studied, methanol proved to be the most influential modifier. A reactive solvent modifier was also evaluated for PAH extraction from reference materials.[166] Two commercially available derivatizing agents (hexamethyldisilane and trimethylchlorosilane) were added prior to SFE with CO_2 and were found to be approximately six times more efficient than CO_2 alone and two and a half times more efficient than methanol modified CO_2 in extracting PAHs. These compounds can increase the yield of both reactive and non-reactive analytes when added to the sample matrix to which the aromatic compounds are adsorbed by disrupting the interactive forces responsible for their strong adsorption.

In addition to the effect of modifier on the supercritical CO_2 extraction efficiencies of PAHs from environmental solids (marine sediment, diesel soot, and air particulate matter), the effect of temperature was also recently investigated.[167] Samples were extracted at 400 atm with pure CO_2 or with CO_2 modified with either methanol, diethylamine, or toluene (all at 10 vol%) at 80 °C and at 200 °C for 15 minutes static followed by 15 minutes dynamic SFE. An increase in PAH recoveries was observed from all three samples by raising the temperature of either the pure or modified CO_2 from 80 to 200 °C, demonstrating that the temperature enhancement was independent of the sample matrix. Methanol was the poorest modifier for all three samples at any temperature and frequently produced no efficiency increase over pure CO_2 extractions. In comparison, both toluene and diethylamine yielded increased recoveries at both temperatures for the air particulate matter, but only diethylamine gave increased recoveries from all three samples.

As well as the use of an organic modifier to enhance the recoveries of PAHs obtained using SFE, fluids other than carbon dioxide have been implemented whose performances are usually compared against that of the more conventional CO_2. These fluids include both pure and modified N_2O and ethane which was used to extract PAHs from urban dust, fly ash and river sediment.[168] N_2O was also used in extracting PAHs (and PCBs) from standard reference sediments and was compared with $CHClF_2$ as an extraction fluid.[169] $CHClF_2$ consistently yielded the highest extraction efficiency because of its high dipole moment, with CO_2 giving the lowest recovery. Also, despite its rather high critical parameters (Tc = 374 °C; Pc = 221 bar), water has been used to remove PAHs from environmental solids (soil and urban air particulates).[170] Extraction was achieved at moderate pressures (*ca.* 50 bar) using water (even though PAHs are low-polarity compounds and typically have low solubilities in water) by raising the extraction temperature which in turn reduced the

dielectric constant. For example, subcritical water at 250 °C has a dielectric constant of 27 when compared to 80 at ambient temperature. Decreasing the polarity of the water by sequentially raising the extraction temperature from 50 °C to 250 °C and finally to 400 °C allowed class-selective extractions of polar organics (chlorinated phenols), low polarity organics (PAHs) and non-polar compounds (alkanes) to be performed.

Frequently in the application of supercritical fluids to removal of PAHs from solids, SFE apparatus has been successfully coupled directly to chromatographic instrumentation. Capillary supercritical fluid chromatography (SFC) with Fourier transform infra red spectroscopy (FTIR) detection has been linked to SFE for the extraction and analysis of PAHs in a coal tar pitch.[171] The variable solvating power of the supercritical CO_2 was used to fractionate the sample with complete separation affected by SFC. Several of the analytes separated in the chromatograph were deposited on a potassium bromide disk for subsequent FTIR analysis. The spectra obtained demonstrate the power of the technique to distinguish between PAH isomers. FTIR has also been used in an off-line SFE configuration for the analysis of petroleum hydrocarbons in soils.[172]

However, most of the work undertaken has been concerned with coupling SFE to GC since this is the most common method for PAH detection from solid samples. The benefits of on-line extraction linked to GC have previously been discussed, with identical methods of coupling SFE to GC employed for PAH detection. Hawthorne and co-workers used a GC with a conventional split / splitless, septumless injection port to link a SFE with a fused silica restrictor.[173] A toggle switch was incorporated into the design to enable the carrier gas to be shut off during extraction. Peak shapes for on-line detection compared well with those obtained from a standard solvent injection. A marine sediment sample was analyzed using both CO_2 and N_2O as extracting fluids, although N_2O was found to yield more rapid extraction of PAHs than CO_2. Carbon black was extracted using a similar technique of coupling SFE to GC, with the PAHs extracted analyzed using a flame ionization detector.[174] Once the analytes had been extracted they were cryogenically focused at the head of the analytical column to ensure no peak shape deterioration. The same authors produced a later paper discussing similar work concerning SFE-GC of PAHs.[175] A comparison of the reproducibility of the peak areas for a SFE-GC extract and a standard split injection was made showing the coupled technique to have the higher precision in almost all compounds investigated. An example of SFE-GC being used to extract an alkane / aromatic test mixture, from an alumina adsorbent in the SFE cell, was shown

by Levy *et al.*[176] CO_2 was used at 375 atm and 30 °C with the analytical column being kept at 30 °C for 8 minutes to enable solute focusing. Once again the peak area relative standard deviation was comparable with a standard injection. The possibility of selective extraction was demonstrated when N_2O was used as extracting fluid where, at low pressures, only alkanes and 1-2 ring structures were extracted. However, at increased pressure (400 atm) higher molecular weight molecules were eluted. SF_6 was also used to show selective extraction of alkanes.

The other most common method of coupling SFE to GC is by using a conventional on-column injection port in which is placed the silica restrictor from the SFE. This technique has been extensively utilized by Hawthorne and co-workers and been shown to successfully remove PAHs from standard reference samples,[177,178,179] and also treated wood, urban dust and river sediment.[180] In all cases, great enhancements in sensitivity were obtained since all of the extracted analytes were focused at the front of the GC column and the subsequent peak shape was as good as a standard injection.

In addition to the PAH applications shown above, a more theoretical investigation of the extraction of PAHs from soils has also been undertaken. Kothandaraman *et al.* evaluated the adsorption and desorption of PAHs from a sandy loam-type soil in the presence of supercritical CO_2.[181] Adsorption data was obtained at three fluid densities, and two temperatures. Unsurprisingly, increasing the density reduced the adsorption level. The non-linear sigmoidal shaped curves produced were represented by regression to the Brunauer-Emmett-Teller (BET) isotherm equation. The addition of small amounts of polar modifier (methanol and water) was found to have an enhanced effect on the removal of PAHs from the soil, with an initial steep decrease corresponding to the first 0.2 % modifier added. Above 0.6 %, further additions of water or methanol had only a minor effect on the adsorption. Desorption was found to be almost complete after 150-200 minutes, depending upon the analyte. A novel process was also proposed for the remediation of soils contaminated with heavy molecular weight organic compounds including naphthalene and phenanthrene (and hexachlorobenzene, pentachlorophenol).[182] Supercritical CO_2 was used to effect the remediation process with the re-adsorption of the pollutants occurring on activated carbon. The desorption profiles of the organics from the soils and the adsorption isotherms of these organics on activated carbon were determined. The desorption profiles from the soils indicated that the organics can be completely extracted in a short time (approximately 1000 seconds). Also, the adsorption isotherms of the

organics on activated carbon showed the adsorptive capacity of the carbon to be very high and approach monolayer coverage.

SFE from an Aqueous Matrix

Despite the often non-polar nature of many of the pollutants already discussed, concern about their presence in both raw and drinking water has necessitated their constant monitoring. However, there has been little research into the use of SFE for extracting analytes from aqueous samples when compared to the vast amount of work which has been carried out in its use for solid samples containing little or no water. This has been primarily due to the problems of retaining an aqueous sample in a conventional "flow-through" extraction cell and, more importantly, to the solubility of water in supercritical CO_2, which is approximately 0.3 %.[183] This can cause restrictor plugging by ice during the supercritical fluid adiabatic expansion and carry-over of water into the collection solvent and ultimately into the chromatographic detection system.

The SFE of pollutants from aqueous media has focused on two main aspects, the use of solid adsorbents and direct extraction. The former has been facilitated primarily using solid phase extraction media through which the fortified aqueous sample is passed. The function of SFE has been to desorb the entrapped analytes from the solid phase extraction media. The direct SFE of analytes from aqueous samples has met with less success because of the length of time for extraction to occur and the possible unfavourable kinetic limitation of the method. However, the simplicity of modifying a conventional extraction cell allows the method to warrant further fundamental investigations.

Use of Solid Adsorbents Prior to SFE

One of the first reported papers in this area was by Wright *et al.*[184] who evaluated SFE from a range of solid absorbents including XAD-2 resin, polyurethane foam and Sphericarb. The versatility of the SFE method was further demonstrated by the use of not only modified and un-modified CO_2 as the supercritical fluid, but also isobutane. The extraction of PAHs indicated that pure CO_2 was ineffective at extracting the highest molecular weight compounds from the spiked XAD-2 resin and polyurethane foam. This is possibly due to the lower solubility of higher molecular weight compounds in the non-polar CO_2. Acceptable recoveries, as compared to Soxhlet extraction, were achieved using either CO_2 + 20 % methanol or isobutane. In a later

publication, spiked water samples were passed through a vessel containing Tenax GC adsorbent (2,6-diphenyl-p-phenyleneoxide) with the aid of a nitrogen flow, prior to elution with supercritical methanol-modified CO_2.[185] The extracted analytes (chlorobenzenes) were trapped in ethanol or directly into a GC injector for on-line analysis. More recently, aqueous samples fortified with 2,4-D and 2,4,5-T, were distributed onto a solid support (Extrelut) prior to extraction with supercritical CO_2.[186] After their immobilization, the samples were derivatized to their methyl esters (with methyl iodide) which allowed their extraction with un-modified CO_2. Conversion to these derivatives from 1 ppm solutions was completed in 30 minutes, with yields of 89 % and 103 % for 2,4-D and 2,4,5-T, respectively. The presence of methanol modifier in the extracting phase was found to reduce its extraction efficiency. In addition, a dynamic mode of extraction, where the derivatizing agent was continually supplied to the extraction vessel was found to be more demanding of reagents and equipment and gave less repeatable results than a simpler static reaction system.

Perhaps more straightforward has been the adoption of solid-phase extraction media in the form of SPE extraction disks (Empore™) which are described in greater detail in section 2.3.2.1. The use of these disks has been favourably demonstrated by several groups of workers. Howard and Taylor,[187] quantitatively removed two sulfonyl urea herbicides from water using C_8 SPE disks. Recoveries of 97.2 % (RSD = 7.8 %) and 93.6 % (RSD = 5.5 %) were achieved for sulfometuron methyl and chlorsulfuron, respectively, using 2 % methanol-modified CO_2. Tang *et al.*[188] extracted PAHs, PCBs, organochlorine pesticides, and phthalate esters from reagent water with Empore disks and SFE elution. The entire procedure was compared with published results from the EPA Method 525(2) in which SPE cartridges and solvent elution was used. After optimization of the SFE operating variables, efficient recoveries were obtained in a faster time compared with the EPA method. Similar work was carried out by Ezzell and Richter who used C_{18} Empore disks to trap five different pesticides and four phthalate esters prior to supercritical fluid elution with CO_2 and modified CO_2.[189] In off-line mode, recoveries were comparable with organic solvent extraction at a 100 ppb concentration, whereas in on-line SFE / SFC studies, recoveries greater than 90 % were obtained for the pesticides studied. C_{18} disks have also been used to extract phenols from water and were compared to the performance of disks impregnated with stryene divinyl benzene (SDB).[190] The relatively high solubility of phenols in water caused break-through of analytes in the C_{18} disks although quantitative recovery was obtained using the SDB disks. In addition, *in-situ* acetylation formed derivatives that were much easier to recover from large sample

volumes (>1 litre) and were much easier to chromatograph than the free phenols. In more recent studies, C_{18} Empore extraction disks have been used to extract a range of 43 semi-volatile organic compounds (including PAHs, chlorinated pesticides, and herbicides) from various waters (tap, river, and ground).[191] Recoveries ranged from 25-154 % depending upon the matrix and the analyte. PAHs were again the subject of a study to optimize the parameters affecting their recovery from water at the low parts-per-billion range.[192] The analytes were deposited on a C_{18} extraction disk prior to elution with supercritical CO_2. The optimum SFE elution conditions were found to be a three-step, 27 minute extraction where the extraction pressure was raised from 2600 p.s.i. to 5100 p.s.i. at 80 °C and a liquid flow-rate of 2 ml min^{-1}. The average recovery for all 16 PAHs investigated was 66 % (RSD 14 %) with lower recoveries obtained with the higher molecular weight compounds. A combined solid-phase extraction (Empore disk) and SFE approach was used to selectively extract surfactants. An alcohol ethoxylate (AE) was selectively extracted from an alcohol phenyl ethoxylate (APE) and vice versa.[193] The selectivity was achieved by changing the density of CO_2 by 0.1 g ml^{-1}. The determined threshold density of the AE was 0.75 g ml^{-1}, while for the APE was 0.85 g ml^{-1}. These small changes in density were sufficient to achieve >90 % selectivity when extracted from Empore disks, and were monitored by capillary SFC. Further use of C_{18} Empore disks was demonstrated with their use in removing APE from an aqueous matrix.[194] An average of 90 ± 4.3 % (for n = 3) recovery was obtained from water samples spiked with 10 mg of APE when they were eluted with methanol-modified CO_2 (10 %).

Direct SFE from An Aqueous Matrix

The first reported method in which analytes were directly extracted from an aqueous matrix was in 1989 by Hedrick and Taylor.[195] In this paper a conventional 8 ml capacity extraction cell was modified so that incoming CO_2 was bubbled through the aqueous sample and the "head-space" atmosphere (CO_2 + extractives) was removed. The demonstration of the effectiveness of this method was reported for the extraction of phosphonates from water. However, no quantitative recovery data was shown. This initial paper was followed with another detailing the extraction of phenols and drugs from water.[196] The total volume of sample and water in the cell was estimated to be around 6 ml. For the phenols studied, the highest reported recovery was 69 %. This recovery (69 %) was obtained at a pressure of 150 atm by passing 50 ml of CO_2 at a flow-rate of 2 ml min^{-1}, *i.e.* for 25 minutes. The same authors have also used the same instrumentation to successfully extract organic bases directly from water.[197] A simple method for the analysis of PAHs in waste water samples was described with on-line detection by SFC.[198] The system used a typical SFC and a "cartridge-like" extractor.

Extraction and analysis of extracts was carried out by introducing the sample directly in the system *via* a sampling valve. This also required no sample clean-up since the extractor functioned as a pre-column and retained co-extractives with the extractor being placed just before the column allowing quantitative transfer of extracts without loss. The PAHs studied (four) were compared against extraction by a conventional liquid-liquid technique and it was found in all four cases that the SFE-SFC method gave higher recoveries.

The design and performance of an on-line liquid / SFE system for the extraction of phenol and 4-chlorophenol from aqueous samples was demonstrated.[199] The system employed a phase separator constructed from either PVDF or Delrin (polymers of the structure -$(CH_2$-$CF_2)n$ and -$(CH_2$-$O)n$, respectively) for the SFE. No deterioration in performance in either phase separator was noted after several months usage. Quantification was achieved using a combined SFE-phase separator with SFC detection. Extraction efficiency was reported to be >85 %. A different approach was more recently used to remove water from the supercritical CO_2 phase after direct extraction from an aqueous matrix. A hollow fibre membrane, which restricts water molecules from entering the stripping medium (CO_2) because the silicone rubber membrane is non-porous and hydrophobic, was utilized in the removal of semi-volatile compounds from aqueous samples.[200] 2 ml water samples containing the analytes of interest (2,4-dimethylphenol, and 2,4-dichlorophenol) were transferred to a reservoir connected to a syringe pump set to deliver CO_2 at 3000 p.s.i. to both the reservoir and extraction cell. The sample was forced through the hollow fibre membrane with the CO_2 simultaneously being introduced into the extraction cell. The CO_2 flowed around the membrane and the analytes were carried out by the high-density gas and collected in propanol. Average recoveries were 74.41 % and 68.85 % for 2,4-dimethylphenol and 2,4-dichlorophenol, respectively, at a sample flow-rate of 0.05 ml min^{-1}.

SFE of Pollutants from Plant and Animal Tissues

The major impetus in SFE related to environmental analysis has been concerned with extraction from soils and related samples. This is not surprising considering the environmental implications of the dispersal of pesticides. Also, from an SFE point of view, the lower moisture content of soils as compared to plant / animal material provides a more compatible matrix from which to extract. Consequently the literature

available is firstly, limited in scope and secondly, almost exclusively related to plant materials.

For obvious reasons, much of the published research into the use of SFE for removal of pollutants from plant matrices has focused on the extraction and analysis of pesticides from crops. SFE of chlorpyrifos methyl, an insecticide, which had been slurry spiked onto ground wheat kernel was reported.[201] Separation and detection using GC and electron capture proved to be ineffectual due to the SFE of co-extractives. Further work included the development of a multi-dimensional chromatography method using LC-GC to effect extraction clean-up. Recoveries using supercritical CO_2 were approximately 64 % even at high pressures (400 atm). However, quantitative recoveries (97.8 %) were achievable using methanol-modified (2 %) CO_2. The co-extractives also caused problems (restrictor plugging) when straw and barley seeds were extracted with supercritical CO_2.[202] The problem was solved by incorporating a post-extraction flush of the restrictor using iso-octane. The efficiency of supercritical CO_2 was investigated for the extraction, at different pressure and temperatures, of fortified grain samples (wheat) containing OCPs, OPPs and organonitrogen pesticides.[203] A Florisil sorbent trap was used to isolate the extracted analytes of interest. Using 20 g samples, extractions were performed between 40 and 80 °C with pressure from 2000-10,000 p.s.i. In most cases, pesticide recovery exceeded 80 % over the range of conditions. A rapid SFE method was investigated for the analysis of organophosphorus pesticides from rice samples.[204] Methanol modified CO_2 was used to extract relatively small samples of rice (7 g). GC with atomic emission detection, used in phosphorus and sulphur modes, was used for quantitation as it can be ten times more sensitive than more conventional detectors. The recoveries obtained compared well against a more common solvent extraction method using methanol. The dilemma concerning the most appropriate commercial SFE instrument for a specific task was regarded in a publication by King *et al.*, who investigated four different instruments for their effectiveness in removing pesticides from various matrices (poultry fat, wheat and soybean oil).[205] The SFE instrumentation employed on this trial was obtained from Hewlett-Packard, Isco, Suprex and Lee Scientific. The study concluded that all three matrices could be successfully employed on commercial SFE instruments. All pesticide recoveries were excellent with acceptable reproducibility of extractions. However, each commercial instrument although consisting of the same components may be better suited to particular methods or to automation.

Often, the higher moisture content of plant / animal materials requires the addition of an *in-situ* sorbent to aid SFE. This has been demonstrated recently by the extraction of

thiocarbamate pesticides from apples using diatomaceous earth (Celite).[206] The effect of adding the Celite was to improve thiocarbamate recoveries by aiding in the immobilization of the aqueous components of the apple matrix. Methomyl had a reported recovery of 84.3 ± 7 % (n = 3) obtained for a total analysis time, including sample preparation and HPLC assay, of 1 hr. The addition of pelletized diatomaceous earth to disperse the sample material and adsorb water has also been effectively demonstrated.[207] The addition of the enhancer allowed the supercritical CO_2 extraction of pesticides and matrix components from both fatty and non-fatty foods which ranged from 95 % water to pure lipophilic oils. Recoveries of over 85 % were achieved for over 30 types of pesticides in diverse matrices such as carrots, lettuce, peanut butter, hamburger, fortified butter fat and potatoes.

As well as the problem of excess moisture in a sample, the matrix may also possess other compounds which co-extract and interfere with their subsequent analysis, namely fatty materials in the case of plant and animal tissues. Adsorbents have been added to these tissue samples for the purpose of in-line clean-up during extraction with supercritical CO_2. Florisil was used at the outlet end of an extraction vessel containing aquatic plants which were fortified at the 5-10 mg kg^{-1} level with organochlorine pesticides.[208] However, when the Florisil was present, recoveries of the pesticides dropped from an average between 89 and 109 % to 56 to 106 %, showing a marked reduction for all pesticides studied. Unfortunately, the use of a solid adsorbent to effect clean-up does have several disadvantages. Highly polar compounds tend to be retained by such adsorbents and are therefore not separable from the fat matrix. Additionally, such adsorbents need to be rejuvenated at the end of each clean-up step and hence are not compatible with an automated system. A new technique was proposed for the clean-up of plant tissues high in fat (corn oil) using gel permeation chromatography (GPC) after SFE with CO_2.[209] A mixture of the corn oil containing several components, bis(2-ethylhexyl)phthalate, methoxychlor, perylene, and sulphur, was extracted with pure CO_2 followed by GPC in both on- and off-line modes. On-line SFE-GPC proved efficient at trapping and fractionating the variety of components with recoveries ranging between 86 to 107 %. No loss of efficiency was reported after continued use of the SFE-GPC system, which was also used as the means of analysis incorporating UV detection.

The modification of either the sample or matrix has been utilized in the removal of extractives from crop tissues. Simultaneous supercritical fluid derivatization and extraction was investigated as an alternative to liquid solvent extraction followed by derivatization for analysis by GC.[210] Addition of a commercial silylation agent (Tri-Sil Concentrate) directly to the sample matrix, enabled extraction of analytes from

samples that were previously exhaustively extracted with conventional SFE with CO_2. Samples studied included coffee and tea. An alternative approach to improve SFE recoveries was suggested by Thomson and Chesney.[211] In this paper 2,4-dichlorophenol, a plant metabolite of the herbicide 2,4-dichlorophenoxyacetic acid, was ineffectively extracted from food crop tissues (straw) using supercritical CO_2. However, the inclusion of a pre-treatment step prior to SFE had a dramatic effect on analyte recovery. The pre-treatment involved acid hydrolysis to disrupt the covalently bound 2,4-dichlorophenol from the plant matrix. However, higher recoveries were still obtained by steam distillation for the field-treated straw samples.

In addition to the SFE of plant samples, several publications consider the use of supercritical fluids to remove analytes from animal tissues. SFE with CO_2 was used for the extraction of PCBs and chlorinated pesticides from biological tissues (fish).[212] Relatively low extraction pressures and temperatures of 100-143 atm and 40-60 °C, respectively, were used to remove the analytes from the matrices which had been mixed with a drying agent (sodium sulphate) prior to extraction. Recoveries ranged between 81-106 % for samples with between 0.3 and 12 % lipid content. Alachlor-fortified lard, bovine liver, and carbofuran-fortified frankfurters were monitored by SFE using an enzyme immunoassay.[213] The nature of the static SFE extraction for the alachlor-fortified lard and bovine liver is particularly novel from an SFE point of view. In each case, sample is loaded into an extraction cell with dry ice. Each sealed cell is then heated, which sublimes the dry ice, yielding supercritical CO_2. After a specified period for static extraction the sealed vessel can be vented under a controlled pressure, through a restrictor. This device allows SFE to be used on-site in a food production facility with the minimum of expertise required. The same group of workers used supercritical CO_2 as a clean-up technique for the separation of organochlorine pesticides from fats.[214] The technique used either an alumina or silica preparative column with a supercritical mobile phase of methanol modified (2 %) or un-modified CO_2, respectively. The method was successfully applied for the extraction of heptachlor epoxide, dieldrin and endrin from chicken fat and lindane, heptachlor, heptachlor epoxide, dieldrin, endrin and o,p'-DDT from spiked lard. Recoveries ranging from 93-111 % compared favourably with conventional column clean-up methodologies. Chicken tissue was again used in a study in the use of supercritical CO_2 for the isolation of sulfonamides using an in-line adsorption trap.[215] Following SFE (10,000 p.s.i. at 40 °C, 4 minutes static prior to a 40 minute dynamic extraction), the analytes were recovered from neutral alumina with HPLC mobile phase (65 % of a 0.05 M phosphate buffer and 35 % methanol) and injected directly onto HPLC columns without the need for post-extraction clean-up. Mean recoveries

of sulfamethazine, sulfadimethoxine, and sulfaquinoxaline from liver, breast tissue, and thigh muscle are 89, 95 and 77 %, respectively, with detection limits lower than 100 ppb.

On-line SFE has been utilized in recovering compounds from animal tissue. Murugaverl and Voorhees developed a method for the on-line SFE / SFC of pesticides (diuron, alachlor, bendiocarb, and carbaryl) in soybean oil and rendered fats which also involved SPE for sample clean-up.[216] Fats, including those from soybean oil, lard, beef fat, and bacon fat were fortified with known amounts of each pesticide (concentration between 0.7-110 ppm). The samples were mixed with three parts of C_{18} sorbent (used to remove lipids co-extracted from the samples which would subsequently interfere with the SFC) and placed in the extraction cell. The samples were extracted with pure CO_2 using a linear density gradient of 0.2 to 0.6 g ml^{-1} over 15 minutes. The SFC analysis was complete in less than 30 minutes with any lipid material still present in the extract eluting after the peaks of interest. SFE has also been directly coupled to GC for the extraction and analysis of PCBs in biological samples (cod liver, cod fillet, and crab meat).[217] The samples were mixed with sodium sulphate, homogenized in a blender, and fortified with PCBs before being added to an extraction cell containing basic alumina at the outlet end. Various densities of supercritical CO_2 were investigated to obtain a useful compromise between the amount of fat extracted and the recovery of PCBs, with the best result obtained using a density of 0.69 g ml^{-1}. The extracted PCBs were cryofocused (-10 to -30 °C) on a length of retention gap before being separated on a 30 m DB-5 GC capillary column. Recoveries obtained ranged from 80 to 96 % depending on the matrix.

2.3.2 Solid-Phase Extraction

Liquid-solid extraction is a popular technique that is used to pre-concentrate and/or isolate liquid samples for analysis. The procedure has been applied in a variety of areas not only to compound concentration but also to sample clean-up, fractionation, and solvent switching. The technique of adsorbing analytes onto solid adsorbent materials has long been used in sample preparation where compounds are partitioned onto materials such as charcoal,[218] and polymeric resins, including XAD-1[219] and XAD-4 (stryene-divinyl benzene co-polymers).[220] Tenax (non-polar polymer) has also proved efficient in retaining environmental pollutants from aqueous samples and has the advantage over the other techniques mentioned that it does not require

preliminary pre-treatment (activation or purification). This may be of use if Tenax is to be used in the field.[221] In all of the examples shown, desorption of analytes is achieved by elution with an appropriate organic solvent.

However, modern solid-phase extraction (SPE) had its beginning in 1978 with the commercial introduction of pre-packed cartridges containing adsorbent material.[222] The concept, similar to low pressure liquid chromatography, is the basis for the design of a practical sample preparation technique consisting of small, disposable extraction columns filled with a variety of sorbents. Before the advent of bonded phases in 1960, silica, kieselguhr (diatomaceous earth), and alumina were used exclusively as normal phase sorbents for the separation of polar compounds from relatively non-polar solvent media. Indeed, Florisil (activated magnesium silicate) is still widely used for the isolation of pollutants,[223] although by far the most common un-bonded sorbent is silica. The polar sites on silica adsorb moderately polar compounds dissolved in organic solvents. Aldehydes, alcohols, and organic halides, dissolved in non-polar solvents such as hexane / di-ethyl ether, are representative compounds for adsorption. These analytes can be eluted from the silica column with solvents having a greater solvent strength than the initial solvents. In general, basic compounds are more strongly retained on the mildly acidic silica, with acids being retained strongly on basic sorbents such as alumina. However, water-soluble organics (carbohydrates, glycerol) are very polar and adhere so tightly to the polar silica sorbent that elution by any solvent is impractical.

The inability of SPE using silica sorbents to extract very polar compounds was overcome in the late 1960's when the process of synthetically bonding silica to form siloxanes was conceived for use with liquid chromatography (see theory section, 2.3.2.1). Although the original intent was the conversion of un-bonded silica to a bonded non-polar phase, both polar and non-polar bonded silicas are now available. In modern SPE, bonded silicas are by far the most popular SPE sorbents with a variety of functional groups being utilized for different applications.

The advent of bonded phases has allowed SPE to become a widely used technique that is utilized in many different analytical disciplines including pharmaceutical and environmental analysis. SPE offers many benefits and advantages over more traditional sample preparation techniques (such as liquid-liquid partitioning) which include, high recoveries of analytes, concentration of analytes often without the need for solvent evaporation, fractionation and some selectivity, ease of automation and a reduction in organic solvent consumption.

2.3.2.1 Theory

SPE Sorbents

In SPE, a choice of adsorbent material is made which is dependent on the nature of the analytes and matrix of interest. At present approximately twenty different sorbents are commercially available to extract both ionic and non-ionic analytes of different polarities.[224] The majority of the SPE sorbents (excluding Florisil and alumina materials) are based on chemically modified silica particles onto which various sorbents are chemically bound. The silica used normally has an irregular particle size of around 40 μm with a pore size of 60 Å giving a surface area of approximately 500-600 m^2g^{-1}. The large surface area allows a great contact area between the sorbent material and the analyte and gives only a low back-pressure. In addition, silica offers a chemically stable environment and gives the system mechanical strength. However, as previously discussed, silica cannot easily be used for the extraction of polar compounds without chemical modification.

Bonded Phases Silica has silanol (Si-OH) groups which may be chemically modified so as to alter the properties of the silica surface. The main way in which silica is modified is depicted in figure 2.8 in which the silica is reacted with a substituted dimethylchlorosilane, with elimination of HCl between a surface silanol group and the silylating agent.[225] Before reaction, the silica is treated with acid (refluxed with dilute HCl). This treatment produces a high concentration of reactive silanol groups at the silica surface, and also removes metal contamination and fines from the pores of the material. After drying, the silica is then refluxed with the substituted dimethylchlorosilane in a suitable solvent, washed free of unreacted silane and dried. This reaction produces a "monomeric" bonded phase, as each molecule of the silylating agent can react with only one silanol group. More complicated surface structures can be produced by changing the functionality of the silylating agent and the conditions under which the reaction is carried out. For example, the use of di- or tri-chlorosilanes in the presence of moisture can produce a cross-linked polymeric layer at the silica surface, as depicted in figure 2.8 (ii). Monomeric bonded phases are preferred, as their structure is better defined and they are easier to manufacture reproducibly than the polymeric materials.

Many other methods have been used to prepare bonded phases. Some examples of these include esterification of the surface silanol groups with alcohols, or conversion of the silanol -OH to -Cl using thionyl chloride, followed by reaction with an amine.

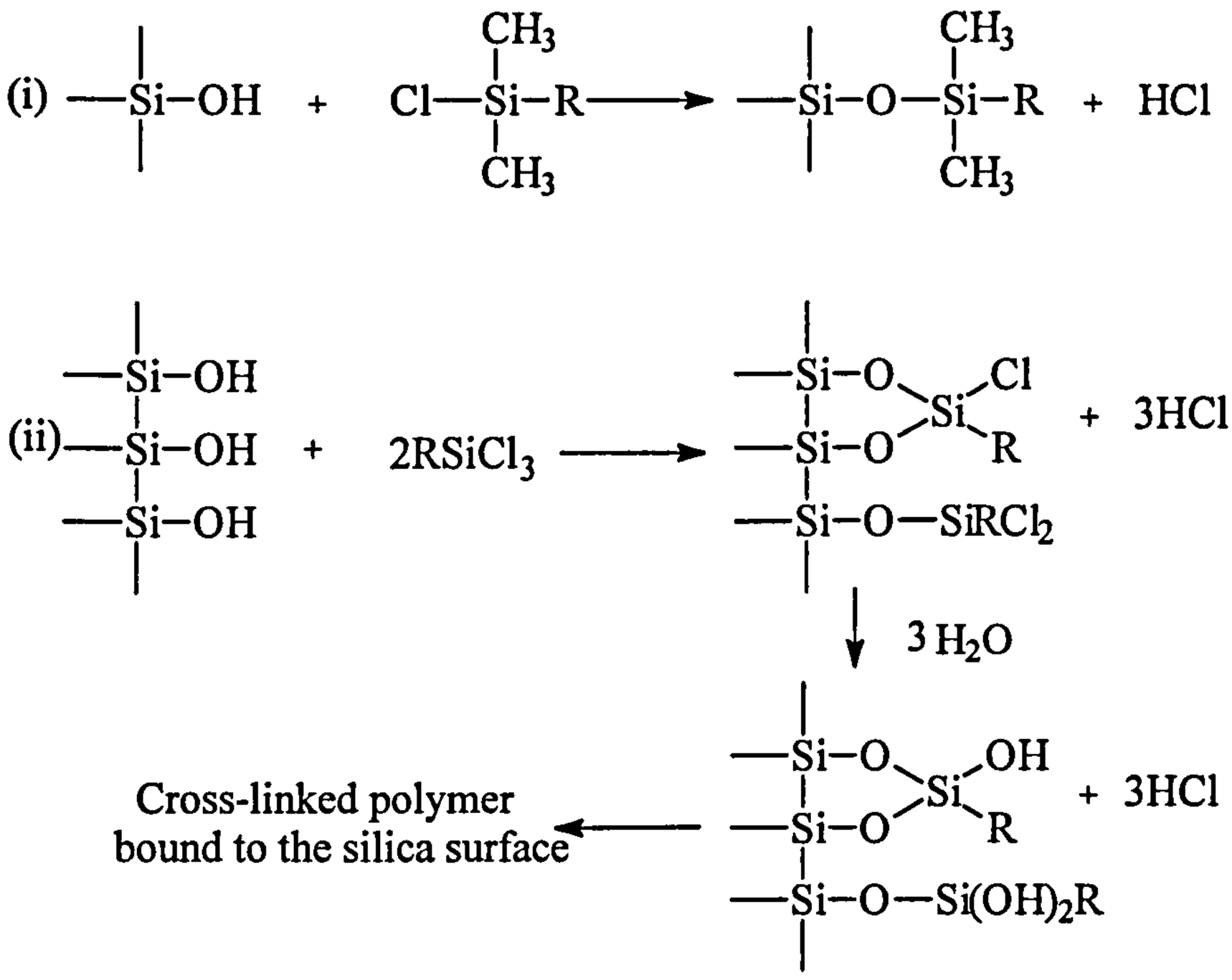


Figure 2.8 Preparation of Bonded Phases. Reaction of silica with substituted chlorosilanes to form (i) monomeric and (ii) polymeric bonded phases.

The sorbent materials bonded onto the silica (*i.e.* the R groups in figure 2.8) include reverse-phase *n*-octadecyl (C_{18}) and *n*-octyl (C_8) phases for the extraction of non-polar analytes and cyanopropyl (CN) and aminopropyl (NH_2) for the removal of more polar compounds. Ion-exchange solid-phase sorbents (*e.g.* benzenesulphonic acid) are also available for the extraction of ionic compounds. Each of these different sorbents exhibits unique properties appropriate for the retention of specific analytes.

In both types of reactions shown above, residual un-bonded silanol groups always remain after the bonding reaction. The presence of un-bonded silanols causes the bonded phase to exhibit heterogeneous surface characteristics, including those due to the attached -R group and those due to the un-reacted silanols. These silanol groups are deactivated by "endcapping" with trimethylchlorosilane, shown overleaf for an octadecyl sorbent (figure 2.9). The potential for competitive adsorption on the hydroxyl sites of an otherwise non-polar surface is thus eliminated or minimized.

Interactions with the Bonded Phase Reversed-phase chromatography refers to any system in which the sorbent is less polar than the mobile phase or sample solution. Octadecyl, octyl, cyclohexyl, butyl, and phenyl substituted siloxanes can be used to extract non-polar and slightly polar analytes from polar solvents. The analyte is then

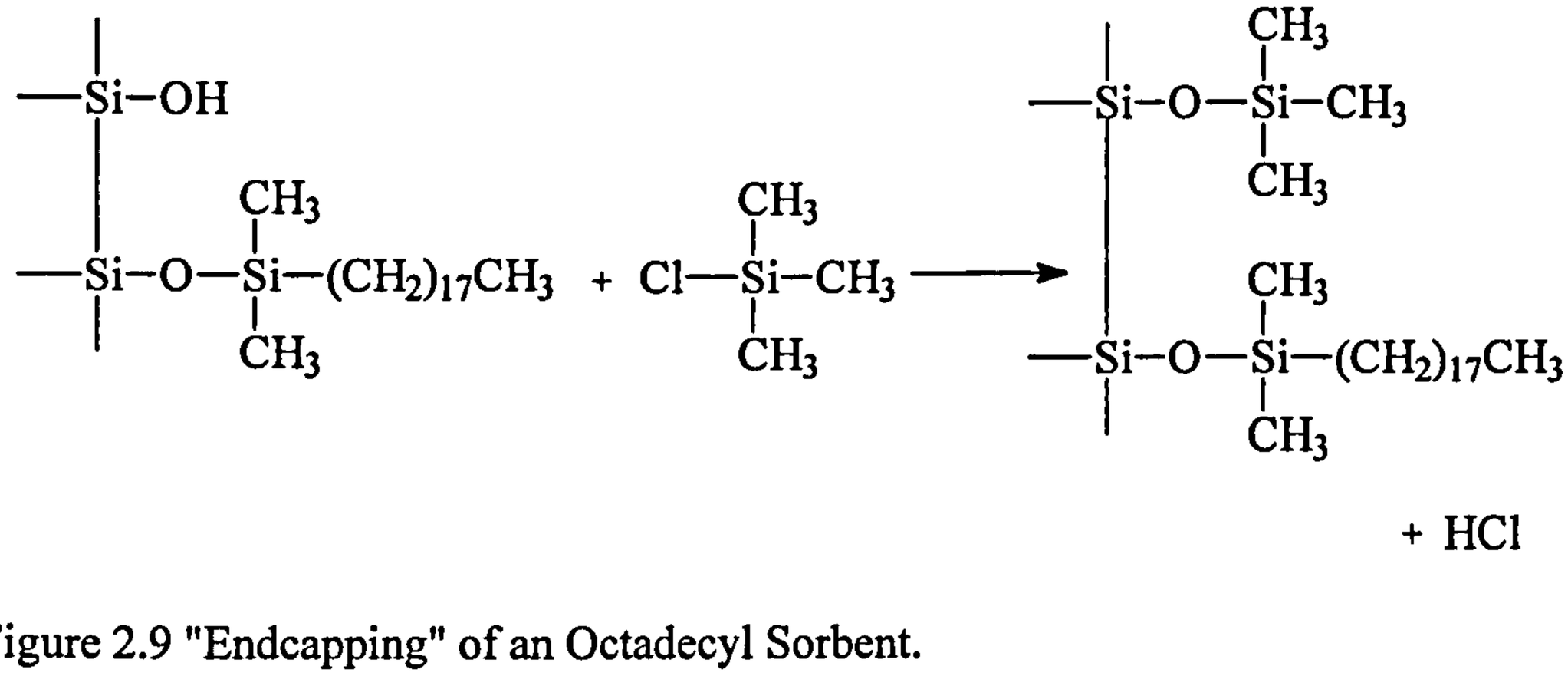


Figure 2.9 "Endcapping" of an Octadecyl Sorbent.

eluted from the column with a less polar solvent. These bonded phases have been used extensively for the trace enrichment of organics from aqueous matrices in environmental analysis. The octadecyl bonded phase has the highest retention for non-polar compounds and is the most non-selective sorbent showing some retention behaviour for many different analytes. However in some instances, the interaction between analyte and sorbent is too great and is difficult to disrupt with a non-polar elution solvent. When this is a problem, retention can be reduced by using a bonded phase with shorter alkyl chains such as octyl, butyl or methyl.

The separation mechanisms of SPE resemble those for low pressure, thin-layer, and high pressure liquid chromatography and occur due to intermolecular interactions between the analyte and the functional groups on the sorbent. These interactions are typically the same as those described in section 1.2.1 and include hydrogen bonding, dipole-dipole, dipole-induced dipole, and dispersion forces. In particular, hydrogen bonding can be an important force on bonded phase surfaces which have residual silanol groups that have not been endcapped. Analyte or interference molecules with the ability to hydrogen bond can therefore interact significantly with the isolated silanols causing strong adsorption. However, for non-polar octadecyl sorbents, the main interaction between analytes and sorbent is due to dispersive forces (van der Waals). The interaction is driven by the hydrophobic nature of the analytes and their insolubility in aqueous solutions. Since polar groups are not significantly involved in this partition, the octadecyl group is relatively non-selective in its attraction for hydrocarbon moieties of solutes.[226]

The Nature of the Hydrophobic Effect The hydrides of small non-metallic elements are gaseous at room temperature. Water is the sole exception and its existence in condensed phases is due to the strength of the O-H...O hydrogen bonds and to the fact

that each water molecule can form four such bonds acting as both a hydrogen donor and receptor. As a consequence, the structure adopted by ice is a tetrahedral one with unfilled space left within the crystal. Pure water has a similar tetrahedral arrangement although the hydrogen bonds within water are weaker than those in solid ice. This means that all of the water molecules in the liquid to be hydrogen-bonded to four neighbouring molecules, but the intermolecular links can be bent and stretched to produce irregular and varied networks.[227]

Hydrophobic substances are defined as those that are readily soluble in many non-polar solvents, but only sparingly soluble in water. In fact the attraction of non-polar groups for each other plays only a minor role in the hydrophobic effect. The hydrophobic effect primarily arises from the strong attractive forces between water molecules, which being isotropically arranged, must be disrupted or distorted when any solute is dissolved in water. If the molecule is ionic or contains polar groups, it can form strong bonds to water molecules, which more than compensate for the disruption or distortion of the bonds existing in pure water; and ionic or polar substances will tend to be soluble in water. No such compensation occurs with non-polar groups and their solution in water is accordingly resisted. In general, aliphatic organic compounds show greater hydrophobicity than their corresponding aromatic compounds with the same number of carbons. This is due to the π-electrons in the aromatic compound which allow stronger intermolecular attraction to water molecules (dipole-induced dipole). The same effect is observed with saturated and unsaturated molecules with the introduction of one double bond being essentially equivalent to removing one CH_2 group from a fully saturated aliphatic chain (although subsequent additions have a smaller effect).

When a hydrophobic compound is introduced in the water matrix there is a disruption in the ordering of the structure. The removal of the molecule is entropically favoured and it will be partitioned into any non-polar environment which it is contact with. Therefore in the presence of the reversed-phase aliphatic chains found in bonded silica adsorbents, hydrophobic compounds are readily removed from aqueous solution.

General Extraction Procedure

There are several key steps which must be followed to achieve efficient SPE. These include initial column pre-conditioning, passage of the sample solution containing the target analytes, removal of interferences during an intermediate wash stage (if required), and finally, elution of the target analytes. The sample and eluting solvents may be forced through the column by positive pressure (syringe), by negative pressure

(vacuum) or by centrifugation. Multiple extraction columns can be processed simultaneously with specially designed manifolds. The overall procedure is shown schematically in figure 2.10, with each stage being detailed below.

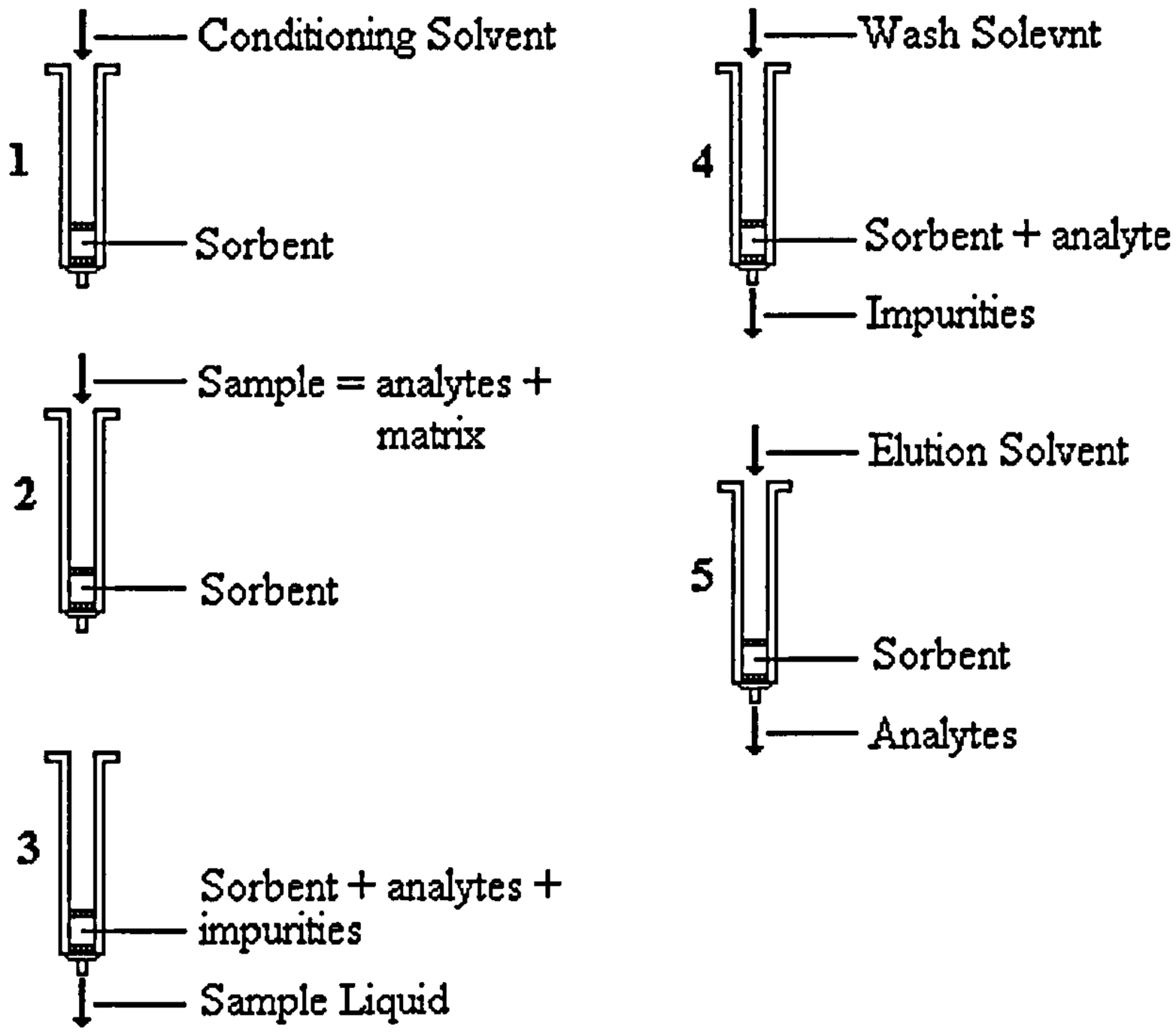


Figure 2.10 Schematic of the Solid-Phase Extraction Process.

Column Conditioning To prepare a column for use, the sorbent is conditioned with an appropriate solvent. In general, methanol or other water-miscible solvents are used in reversed bonded phase applications. The methanol solvates the non-polar groups of the reversed-phase sorbents, but is also miscible with aqueous solutions used in reversed phase extractions. After conditioning of the column with methanol, the column is conditioned with a solvent similar to the matrix solution to remove the excess methanol and to prepare the column to receive the sample. With aqueous extractions, the excess methanol is usually removed by the same volume of distilled water. After the addition of the distilled water it is imperative that the column is not allowed to dry before the addition of the sample and the sample is usually added directly on top of the distilled water wash.

Sample Extraction Once pre-conditioning has been achieved, the sample solution is passed under vacuum through the columns at a flow-rate of approximately 5 ml min^{-1} or less. In the conditioning stage, flow-rates are not of great importance as long as sufficient interaction time between sorbent and solvent is allowed. However, in the

sample extraction stage, too high a flow-rate may cause "break-through" of analytes from the sorbent leading to inefficient extraction. Conversely, if the flow-rate is too low, large sample volumes (1-2 litres), commonly used in environmental sample preparation to achieve the detection limits required, may take several hours to extract. However, the optimum flow-rate used does depend on the size of the cartridge and the amount of sorbent packing it contains and if large samples are to be extracted, large sorbent areas offer faster extraction times. Often in aqueous extractions a small amount of methanol (*ca.* 5 ml) is added to the sample, prior to extraction, to act as a bonded phase wetting agent.

Removal of Interferences The non-selective nature of many reversed-phase sorbents means that they tend to adsorb interferences present in the sample matrix as well as the target analytes. These interferent compounds, if eluted with the target analytes, can affect the efficiency of the subsequent chromatographic analysis technique. However, SPE allows an intermediate washing stage to be performed with a specific solvent that elutes the interferences but does not remove the target analytes. The solvent chosen is dependent on the nature of the interferences but obviously must have no affinity for the target analytes.

Analyte Elution After washing is completed, the column is usually allowed to dry under vacuum to remove any excess solvent prior to addition of the final elution solvent. A collection tube is then inserted below each cartridge and appropriate solvents are added and forced through the columns. The analyte eluates are collected and analyzed directly, or evaporated and then reconstituted in an appropriate solvent for further analysis. If the elution solvent has been properly selected, analytes can be quantitatively removed with approximately two void volumes (the sum of the volume between the sorbent particles and the volume within the pores of the sorbent) of solvent. The void volume on most sorbents is between 1.0-1.2 μl mg^{-1} of sorbent.[226] Typically, 2 x 100 μl is sufficient to elute analytes from a 1 ml column.

Disadvantages of Column SPE

SPE using cartridge columns has many advantages over traditional liquid-liquid partitioning already discussed, including the possibility of automation and a reduction in the amount of organic solvents used. Unfortunately, column SPE also has some inherent disadvantages. The narrow-bore geometry of the sorbent packing does not allow fast sample flow-rates and therefore reduces the speed of extraction. In addition, if the aqueous sample to be extracted is a pore water or groundwater, more likely to contain particulate matter, then this can cause the sorbent to become blocked, further

increasing extraction time. In fact, even clean drinking water samples can taint the sorbent with some amount of filtered material. Samples can be pre-filtered to remove particulate matter but this again is time-consuming and may lead to losses of target analytes which have become partitioned on the solid matter. As well as the problems with "dirty" samples, the construction materials of the disposable SPE extraction columns contribute to the impurity interference levels, as plasticizers and oligomers are extracted from the polymeric column and frit components. Junk *et al.* demonstrated that these components limit the practical detection limits obtainable for the components of interest and were identified as alkanes, alkenes, plasticizers, and antioxidants.[228] However, these problems may be overcome by the use of glass columns (at a much increased cost per extraction) which offer a much "cleaner" surface less likely to cause interferences.

Solid-Phase Extraction Disks

A novel material for separations has been developed which has overcome many of the problems associated with conventional column SPE. Solid-phase extraction disks marketed under the trade name *Empore*, consist of chromatographic particles enmeshed in a network of PTFE fibrils to form a strong, porous sheet or "membrane". The membrane can have a variety of sorptive or reactive properties, which are determined by the characteristics of the particulates chosen.[229] The use of hydrophobic octadecyl- or octyl-bonded silica particulates for reversed-phase extractions from aqueous matrices has, by far, been the most common use for the new technology. The disks are used in conjunction with conventional glass filtration apparatus and therefore do not require that the sorbent be contained in plastic columns, removing the problem of column interferences. In addition, the membranes are available in 47- or 25-mm disks (the construction of the disks is described in greater detail in the instrumentation section) which allow far greater flow-rates and a reduced incidence of blocking due to particulate matter. The disks, as well as being used for conventional SPE, have found many uses in the field of SFE where they are used in the indirect extraction of aqueous matrices due to their ability to be simply rolled and placed in a SFE extraction cell for supercritical fluid elution.

Empore extraction disks are used in exactly the same way as SPE columns with the exception of the addition of a small amount of concentrated hydrochloric acid (approximately 1 ml) to the sample prior to filtering. This has been shown to reduce the time taken to filter an aqueous sample by partially dissolving previously insoluble salts present in the sample.[229] In the study, XRF data indicated the presence of a significant amount of magnesium, aluminium, sulphur and calcium which were

present on the un-treated disk after filtering, but only present in trace amounts after the sample was acid treated.

Unfortunately there is one problem which is inherent in all SPE products which are commercially supplied. Once a commercially obtained disposable SPE cartridge has been successfully utilized to extract analytes, switching brands to that of a different manufacturer's is not recommended. Interchange of bonded phases from different suppliers has been found to lead to spurious results.[226] This is principally due to the source of silica for the preparation of the bonded phase, the carbon loading, the bonding chemistry (mono- or tri-functional derivatives) and endcapping chemistry which all affect the surface characteristics of the columns and therefore the retention behaviour of analytes. Thus, if uniform performance is to be obtained, one manufacturer must be used consistently. In addition to manufacturer-to-manufacturer variation, batch-to-batch variation from the same supplier has also been a problem with SPE.

2.3.2.2 Instrumentation

Column Solid-Phase Extraction

One configuration of a disposable column, pre-packed with a particular sorbent, or bonded phase is shown in figure 2.11. These polypropylene columns are available

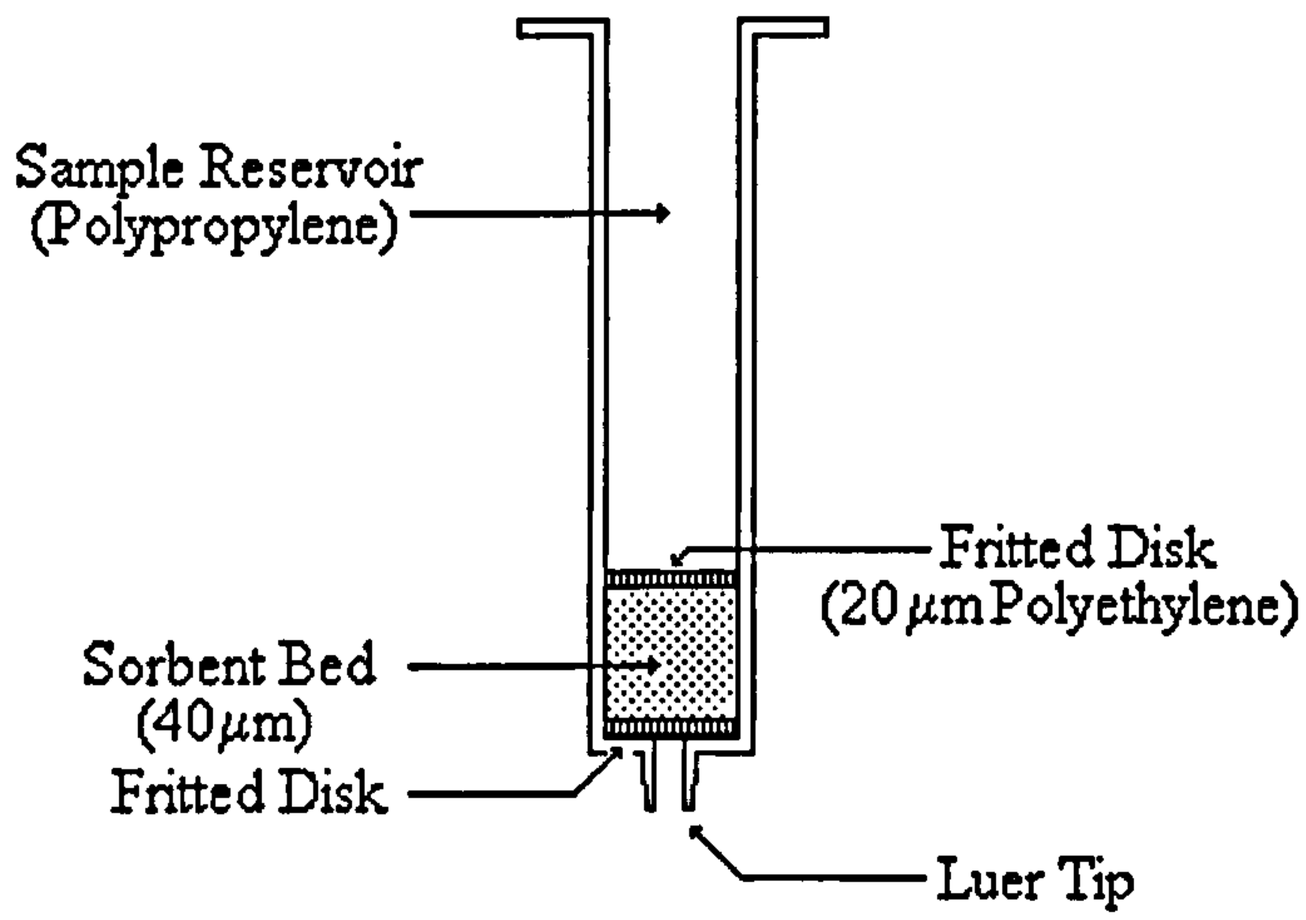


Figure 2.11 A Disposable Extraction Column.

commercially packed with 100, 200, 500, 1000 or 2000 mg of sorbent sandwiched between two 20 μm polyethylene frits. The luer tips are designed to fit into standard vacuum manifolds for use with SPE and allow columns to be placed in series with

each other by using an adaptor. Sample volume capacities are between 1, 2, or 6 ml with large volume samples (up to several litres) being added to the column *via* detachable reservoirs. Solutions are usually aspirated through the columns under vacuum.

Analyte capacity of sorbents and bonded silica gels have been estimated as 10-20 mg of analyte per g of packing.[230] The capacity stated is in terms of pure compounds and if other compounds in the sample are retained by the column, the capacity for the analyte is reduced proportionally to the amount of competing compound present. Analyte "break-through" will occur if analytes pass through the column un-retained and is defined as the point at which the sorbent becomes saturated and can no longer retain additional analyte. Nonetheless, the capacities are far above the analyte concentrations in practical environmental analysis and break-through normally only occurs when high flow-rates are used or when large volumes of sample are passed through the column.

Disk Solid-Phase Extraction

The SPE membranes utilized for extraction are manufactured solely by 3M under the trademark *Empore*. A brief description of the disks follows.

They are available as 47 and 25 mm diameter disks and are nominally 0.5 mm thick. A typical composition is 90 wt.-% octyl- or octadecyl-bonded silica particles (8 μm, 60 Å pore size) and 10 wt.-% fibrilated PTFE. The PTFE comprises less than 1 % of the total surface area of the composition and the open pore volume is *ca.* 60 vol.-%. Each 47 mm disk weighs approximately 500 mg.

The SPE disk can be considered to be a large-diameter, short-length extraction column. The use of smaller particles and uniform packing in the disks provides an efficiency equivalent to that of the relatively small diameter packed extraction cartridges which normally contain 40 μm particles to allow a reasonable flow without excessive back-pressure. The highly uniform packing also eliminates the channelling often experienced with packed columns.[229]

2.3.2.3 Environmental Applications

The high volume of drinking water samples that are routinely screened for the presence of pesticides and herbicides in environmental laboratories has prompted an

investigation into other sample preparation techniques that require less analysis time and allow easier automation than traditional liquid-liquid extraction. Solid-phase extraction is fast becoming the method of choice for environmental water analysis since it offers a high throughput of samples combined with ease of automation and a reduction in organic solvent usage. Both cartridge and disk forms of SPE have been utilized in pesticide analysis, although the former is still the most popular if large numbers if samples are to be determined because of the possibility of automation.

Many different classes of pesticides have been studied using SPE as the enrichment technique but probably the most common is that of the organochlorine pesticides (OCPs). A great deal of research has been conducted into SPE of organochlorine pesticides because they feature in the UK's Department of the Environment's "Red-List"[231] and its equivalent in other countries. In addition, OCPs are relatively non-polar and therefore are readily extracted using long chain hydrocarbon sorbent phases. "Home-made" glass micro-columns filled with octadecyl (55-105 μm) sorbent have been successfully used to extract twelve different OCPs including lindane and DDT.[232] The glass columns were favoured over commercial plastic cartridges because the latter have been shown to produce foreign peaks when the extracts were analyzed using gas chromatography with highly sensitive electron capture detection (GC-ECD). An average recovery of over 90 % was obtained for spiked water samples at 0.1 ng ml^{-1}. Reverse-phase C_{18} cartridges have also been used to extract OCPs from human serum or plasma with GC-ECD analysis.[233] SPE, using both C_{18} and XAD-2 solid phases, was compared with conventional liquid-liquid partitioning for the determination of 16 OCPs (US EPA priority) spiked into water at a concentration range of 20 ng l^{-1} to 20,000 ng l^{-1}.[234] The C_{18} solid-phase was found to have several advantages over the XAD-2 phase including a high recovery for a lower amount of phase, elution of OCPs in a narrow band (resulting in a reduction in the amount of solvent required for elution) and no necessity for an equilibration period required for the XAD-2 (because of a slower desorption rate). Recoveries for C_{18} extractions were found to be about 85 % with the exception of heptachlor and aldrin. Solvent extraction showed better recovery in concentrations higher or equal to 200 ng l^{-1} for all pesticides, but required far greater time and utilized large volumes of solvent. However, the SPE method was shown to achieve greater sensitivity than the solvent extraction, even after evaporation of the solvent extract (dichloromethane switched for hexane).

Although SPE is primarily used as an extraction medium, it has been shown to be of use in the clean-up of extracts prior to chromatographic analysis. C_{18} and Florisil SPE

were used as an alternative to Attagel clean-up procedures for the determination of halogenated organic pesticides in raw agricultural crops.[235] The alternative clean-up procedures have been sought to substitute Attagel, which uses benzene as the elution solvent, and to minimize matrix interference and adsorption of pesticides onto the Attagel. Four different matrices (broccoli, carrot, celery and orange) were spiked with a total of 44 pesticides at levels between 0.2 - 1.6 ppm. After extraction of the food with acetonitrile and pre-concentration (the acetonitrile was reconstituted in benzene for the Attagel clean-up), the extracts were taken through the clean-up procedure using either C_{18}, Florisil or Attagel sorbents. 7 ml of water was added to the concentrated extract (3 ml) for the C_{18} SPE procedure, and the extract was passed through a pre-conditioned C_{18} cartridge. The pesticides were then eluted with 2 ml of hexane or a mixture of hexane and acetone depending on the nature of the pesticides, and analyzed by GC with electron capture detection (identification of the peaks was performed using GC-MS). Low recoveries, between 40 and 83 % were obtained from the Attagel clean-up whereas C_{18} showed recoveries ranging from 81 to 132 %, depending on the pesticide and the matrix. However, disadvantages are reported in using a C_{18} clean-up procedure including, greater time required for the whole method and the presence of water droplets in the cartridge if an extended drying period was not used after the passage of the extracts.

Organochlorine pesticides have also been successfully extracted using membrane extraction disks (Empore). The extraction disks have been used because of their advantage over cartridge SPE when filtering large volumes of sample. Spiked river water containing five different OCPs (adjusted to pH 2.0 using hydrochloric acid and containing 5 ml of methanol) was filtered through a pre-conditioned C_{18} Empore disk prior to elution with ethyl acetate and dichloromethane.[236] After evaporation, the extracts were found to contain approximately 100 % of the OCPs spiked into the river water sample, at the sub-ppb level. The recovery was reduced at lower concentrations (ppt). The large surface area of SPE disks is especially useful when filtering aqueous samples which contain solid materials that can easily block the frits in a SPE cartridge. McDonnel *et al.* investigated the use of Empore extraction disks for the removal of OCPs from natural waters which may contain particulate matter.[237] In addition, in order to obtain sufficient concentrations of analytes to detect, large volumes of sample (10 litres or more) were required to be extracted. Here extraction disks have great advantages over cartridge SPE. When extracting such large volumes of sample, several interferences were detected when using GC-ECD analysis. These interferences were separated by selective elution from the disk which was not possible using conventional column chromatographic techniques. The recoveries obtained

from Empore disks were found to be comparable with those obtained from liquid-liquid partitioning with dichloromethane.

Solid-phase extraction disks have been successfully utilized in removing other classes of pesticides from aqueous samples. Empore disks with C_8 bonded phases were used to remove several different classes of pesticides from soil leachate, including organophosphorus pesticides (OPPs).[238] The pesticides were added to a laboratory leachate at a concentration of 1.0 ppm. A sample volume of 300 ml was filtered through a pre-conditioned disk with the final elution using ethyl acetate. After rotary evaporation, the samples were analyzed using GC with nitrogen-phosphorus detection. It was found that poor recoveries were obtained when extracting large sample volumes that had not been filtered prior to extraction to remove clay particulate matter. Upon filtering, recoveries of certain pesticides improved by around 20 - 30 %. Filtering also had the advantage of reducing the analysis time since flow-rates through the Empore disk were increased. Overall, recoveries of the different pesticides ranged from 61.5 - 94.2 % after filtration. Organophosphorus pesticides have been shown to be quantitatively extracted using Empore disk technology in an on-line arrangement.[239] OPPs were concentrated from aqueous samples (both tap and river water) onto three 0.5 mm thick extraction disks consisting of C_{18} or XAD sorbent material. The layers were then dried with a stream of nitrogen. Desorption of the analytes was achieved with ethyl acetate with the extract being directly introduced into a retention gap, where the solvent was allowed to escape through an exit valve once the GC inlet pressure had been raised. The solvent remaining in the transfer capillary was removed by a small helium purge flow. The final analysis was carried out by GC with nitrogen-phosphorus detection. Using only 2.5 ml of sample, OPPs were detected in river water at lower concentrations than the level of 0.1 $\mu g\ l^{-1}$ set by the EEC directive for drinking water.[240]

The use of non-polar C_{18} and C_8 SPE bonded phases is not purely restricted to extraction of non-polar analytes. Several publications illustrate their use in removing more polar compounds such as carbamate insecticides from aqueous samples which indicates their non-selective extraction capability. de Kok and co-workers studied the effectiveness of C_8, C_{18} and special low-carbon C_{18} sorbent materials for the extraction of twenty N-methylcarbamate insecticides from surface waters.[241] The results show that generally, there was no difference between recoveries obtained from the C_8 or C_{18} sorbent cartridges used. This result is surprising since C_8 packing material has been shown to give significantly higher recoveries when compared to C_{18}. This behaviour has been explained by selective sorption of the polar carbamates

on the free silanol groups of the silica, which are more accessible on the C_8 than the C_{18}-bonded material.[242] However, the highest overall recoveries were obtained using the low-carbon C_{18}-bonded silica which is specially designed for extraction of polar metabolites of pharmaceutical products. 100 ml samples of surface water were filtered through 500 mg cartridges. Elution was obtained using acetonitrile giving recoveries which ranged from 76 - 111 % for both spiking levels (0.1 and 1.0 μg l^{-1}) with relative standard deviations between 0.5 - 7.3 %, depending on the concentration of the spiked carbamates. C_{18} Empore extraction disks have also been used in an on-line arrangement for the trace enrichment of several classes of pesticides, including carbamate insecticides in drinking water.[243] As with the example above, HPLC with post-column fluorescence detection was used for the analysis of the extracts. Although an on-line configuration was used, the fortification levels for the pesticides were higher than the previous example and ranged from 0.2 - 5 μg l^{-1}. Recovery was found to be dependent on both the fortification levels and the volume of sample passed through the Empore disk. At the highest concentration level and lowest sample volume (250 ml), using UV detection, recoveries ranged from 62 - 95 % depending on the pesticide. However upon using post-column fluorescence detection, sample volumes as little as 10 ml were required to achieve limits of detection between 0.005 - 0.040 μg l^{-1}. One additional advantage of the on-line arrangement is that the Empore disks may be repeatedly re-used without any significant deterioration in performance.

Perhaps the most widely used application of SPE in environmental analysis is in the trace enrichment of triazine herbicides from water samples. This is due to the increasing wide-scale usage of triazines and therefore increasing requirement for their determination in aqueous samples. There are many publications illustrating the successful use of SPE. Examples of early research include work by Popl *et al.* in 1983 where seven different triazines were pre-concentrated on a macroporous polymer sorbent (1:2, Separon SE50/50 mixed with ground silica) with elution using acetone or methanol.[244] Other studies include the removal of triazines from natural waters including surface water[245,246,247,248] ground water[249,250,251,252] and sea water.[253] Obviously there are many other examples where triazines have been successfully extracted from drinking water and tap water[254,255,256,257] which is routinely screened for the presence of triazine herbicides.

C_{18}-bonded silica is almost universally utilized for the successful extraction of triazines from water, however, there are several instances where certain triazines are not quantitatively recovered using this system. In particular, desethylatrazine and desisopropylatrazine (chlorotriazine break-down products of atrazine) are often not

extracted efficiently.[249,251,252,258] Cassada *et al.*[252] investigated the influence of sample volume on the recovery of atrazine, desethylatrazine and desisopropylatrazine from three volumes of water (200, 400 and 800 ml). As the sample volume increased it was noted that the recovery of atrazine remained within experimental error (95.1 %) whereas the recovery for desethylatrazine decreased from 93.1 % to 89.0 % to 32.1 % and for desisopropylatrazine from 41.4 % to 24.0 % to 12.8 %. These results were also in agreement with Thurman *et al.*[249] In addition, Chiron *et al.*[258] determined the break-through volume of simazine, atrazine, desethylatrazine and desisopropylatrazine at two different volumes (50-150 ml) of fortified water samples (0.3 ng ml^{-1}). The results indicated that the break-through volumes, based on ten Empore disk extractions, were 8 ml for desisopropylatrazine, 70 ml for desethylatrazine and greater than 150 ml for simazine and atrazine. The break-through volumes were also mirrored in the recovery data obtained using ten Empore disks. For a 50 ml sample the recoveries were 60 % (desisopropylatrazine), 90 % (desethylatrazine), 95 % (simazine) and 97 % (atrazine). However, for the 150 ml sample the triazine break-down products gave even lower recoveries of 17 % (desisopropylatrazine) and 55 % (desethylatrazine) as compared to simazine and atrazine which maintained their high recovery (87 % and 92 %, respectively).

2.3.3 Solid-Phase Microextraction

Although extraction techniques that use little or no solvent have been available for some time, only recently have solvent-free sample preparation techniques begun to attract widespread attention, primarily because of regulatory pressure to reduce the use of toxic organic solvents. Solvent-free operations can be classified into two main categories:

Gas-Phase Extraction This category includes static headspace sampling, purge and trap, and supercritical fluid extraction. Static headspace analysis is perhaps the simplest form of solvent-free sample preparation and has been widely used to analyze volatile organic compounds (VOCs) in food, beverages and other samples.[259] The technique is popular because the extracting phase (typically air or nitrogen) is compatible with most instruments. Here the sample is equilibrated with its headspace and a small volume of the headspace is then directly introduced into a gas chromatograph (GC) for analysis. However, because of the lack of any concentrating effect, headspace extraction suffers from low sensitivity. Also, it cannot achieve exhaustive extraction and calibration is often difficult. Dynamic extraction, more

commonly known as purge and trap, uses a dynamic partition principal and allows quantitative recovery of VOCs.[260] The technique uses gas chromatographic carrier gas, which is bubbled through an aqueous sample, to purge VOCs from the matrix. The analytes are then focused onto a cold trap or suitable sorbent prior to GC analysis. Disadvantages of purge and trap include the possibility of foaming, cross contamination and purging flow-rates which are incompatible with on-line analysis. Although headspace techniques are limited to VOC analysis, they can be utilized to determine less-volatile analytes and solid matrices by heating the sample. Thermal desorption at increased temperatures allows less-volatile compounds to be partitioned into the gas-phase more readily. The final gas-phase method is that of supercritical fluid extraction which has already been discussed in this chapter.

Sorbent Extraction The concept of using a sorbent material to extract organic compounds from aqueous samples is extensively used and is most commonly seen in solid-phase extraction (SPE), discussed in section 2.3.2. As already mentioned, SPE has several attractive features compared with liquid-liquid partitioning including its simplicity, low running costs, possibility of use in the field, ease of automation and the relatively small solvent demand. The advent of solid-phase extraction disks (or Empore disks) has further improved extraction efficiency and helped to reduce the problem of plugging. However, SPE does have some limitations, such as interaction between sample matrix and analytes which results in low recovery and the plugging of the cartridge frits or the sorbent pores. This in turn may lead to low flow-rates and a reduction in break-through volumes and sorbent capacity. Empore disk technology has reduced these negative effects but still does not account for batch-to-batch variations and high blank values. In addition, SPE still requires the extracts to be concentrated by evaporation prior to analysis and therefore cannot be used for VOC analysis since it is limited to semi-volatile compounds whose boiling points are substantially higher than that of the solvent.

One solution to these limitations is to improve the geometry of the sorbent by coating it onto a fine rod such as a fused-silica fibre. The cylindrical geometry of this "solid-phase microextraction" (SPME) system allows for the rapid mass transfer during extraction and desorption, prevents plugging and improves handling and introduction into analytical instruments.[261]

2.3.3.1 Theory

SPME consists of two processes: partitioning of analytes between the coating and the sample and desorption of the concentrated analytes into an analytical instrument. In the first process the fibre is exposed to the sample and the target analytes are extracted from the sample matrix onto the coating. The fibre with the absorbed analytes is then transferred to the instrument for desorption, which is followed by separation and subsequent analysis. Generally, SPME has been used to extract organic compounds from various matrices such as air, water and soils with desorption and analysis being carried out by gas chromatography. For these applications, a fused-silica fibre coated with a gas chromatographic stationary phase, such as poly(dimethylsiloxane), is used. The techniques required to produce the fibres have been well developed for manufacturing optical fibres and the fused-silica fibre itself is chemically inert and very stable even at high temperature.[262]

The principle behind SPME is the partitioning of analytes between the sample matrix and the extraction medium. If a liquid polymeric fibre coating is used, the amount of analyte absorbed by the coating at equilibrium is directly related to its concentration in the sample and depends on the partition coefficient, K of the analyte between the coating and the matrix.[263,264] Typically, the coatings used in SPME have a strong affinity for the analytes of interest and therefore K is usually large, which means that SPME has a very high concentrating effect leading to good sensitivity. However in most cases, the partition coefficient is not large enough to lead to the total exhaustive extraction of analytes from the matrix. Instead, SPME, like static headspace analysis, is an equilibrium sampling method and, through proper calibration can be used to determine the concentration of target analytes in a sample matrix. The speed of extraction is controlled by the mass transport of the analytes from the sample matrix to the coating. This process involves convective transport in an air or liquid sample and diffusion of analytes in the coating. In direct SPME sampling, the mass transfer rate is determined by the diffusion of analytes in the coating if the sample matrix is perfectly agitated.[263]

When the mass transfer is determined by the diffusion of the analytes in the coating, for most analytes equilibrium is achieved in < 1 minute. This rapid extraction is achieved because the coating is very thin, typically between 5 and 100 μm. In principle this rapid extraction rate may be obtained, however, in practice only for gaseous samples because of large diffusion coefficients. For aqueous samples this case may only be achieved when using very vigorous agitation methods such as

sonication.[265] If normal agitation techniques are used (magnetic stirring), the equilibration time is likely to be much longer and is determined by diffusion through a thin static aqueous layer adjacent to the fibre. This layer is difficult to remove even when water is stirred vigorously to enhance mass transfer of analytes and the analytes must still pass through this static layer before they can be absorbed onto the fibre coating.

Two different types of sampling are available in SPME, depending on whether the fibre is immersed into the aqueous sample. Headspace sampling is useful if the sample of interest contains appreciably large amounts of solid material where direct sampling would not work well. Therefore SPME has the capability to extract analytes from virtually any matrix. However, only those analytes that are easily released into the headspace above the sample are capable of being analyzed. Thus, volatile compounds which are released by the matrix relatively easily are ideal for headspace sampling. For semi-volatile analytes, the low volatility and often high molecular size may slow the mass transfer from the matrix to the headspace and in some cases kinetically controlled desorption can also limit the speed of extraction. As in all extraction techniques, when the matrix adsorbs the analytes more strongly than the extracting medium, the analytes partition poorly into the extraction phase. The amount of extraction phase available for absorption is limited in SPME, therefore the extraction will have a thermodynamic limitation (the partition coefficient is too small, resulting in poor sensitivity). If the opposite is true and the coating has a stronger ability to absorb analytes than the matrix, then in a relatively short time period a substantial amount of analyte will have been extracted by the fibre coating, and only kinetics plays an important role during extraction. One of the most efficient ways to overcome kinetic limitation is to heat the sample which increases the vapour pressure of the analytes, provides the energy necessary for analytes to dissociate from the matrix and simultaneously speeds up mass transport of the analytes.

For aqueous samples, the headspace-water partition coefficient is directly related to the analytes volatility and hydrophobicity and is commonly quite small. Thus, the capacity for the headspace to trap analytes is also small. As a result, the sensitivity of headspace SPME is almost the same as that of direct SPME and for volatile compounds in water, headspace SPME sampling is faster than direct SPME. However, for semi-volatile analytes in aqueous samples, headspace SPME is not an efficient technique since the analytes lack sufficient volatility and direct extraction must therefore be performed. Mass transfer is increased by agitating the sample, however, the use of magnetic stir bars is not without inherent problems including a

much higher possibility of cross-contamination, the inability to accurately set the fibre depth in the sample and the practical considerations of the use of such equipment in the confined spaces of an automated SPME assembly. One disadvantage in direct SPME is the reduction in the lifetime of the fibre when compared to that when using headspace analysis.

SPME has two basic functions: extracting analytes from a matrix and desorbing them into an analytical instrument (usually a gas chromatograph). The two stages will be described separately.

Extraction

The most important experimental parameters (sensitivity, accuracy, precision and speed of extraction) in SPME are determined mainly by the extraction stage. The extraction efficiency and thus the sensitivity of SPME depends on the analyte affinity towards the fibre's sorbent coating. Different groups of analytes can be extracted by different types of sorbent, and a variety of sorbents have been used in SPME. The normal concept in organic analysis is "like-dissolves-like" and this principle is followed in SPME analysis. Polar coatings such as polyacrylate are used to extract polar compounds such as phenols, whereas non-polar coatings such as poly(dimethylsiloxane) retain hydrocarbons efficiently. The range of application of SPME is discussed later in section 2.3.3.3.

Sensitivity Several factors can influence the sensitivity of a SPME extraction including, the volume of coating and its characteristics, heating of the sample, cooling of the fibre and the derivatization of target analytes or the modification of the sample matrix. The amount of analyte extracted by the fibre is directly proportional to the volume of the coating and thus the sensitivity improves as the volume of the coating increases (by increasing the thickness of the coating or the length of the fibre). In addition, in this equilibrium based extraction technique, both the matrix and sorbent fibre coating are competing for analytes and a strong affinity of coating for the target analytes is crucial.

Derivatization has been used to alter the affinity of the analyte for the coating. An example is the use of derivatizing agents to convert polar phenols to acetate derivatives which have a greater affinity for non-polar coatings.[266] This derivatization is performed in the actual aqueous sample. Just as the analyte may be chemically altered to suit the fibre coating available, the matrix may also undergo a change in its nature to facilitate extraction. By adding salt (NaCl or $NaSO_4$) to an aqueous sample,

the ionic strength of the sample may be increased, thereby increasing the partitioning of organic compounds (not ions) into the polymeric coating. Also, because neutral forms of analytes are more readily partitioned into the coating, the pH of the aqueous samples must be adjusted to prevent ionization of the analytes.

If the sample of interest is not a liquid but a soil or similar substance then clearly direct SPME cannot be performed. The target analytes must be first released into the headspace before absorption onto the fibre coating can be achieved. The degree to which the analytes are released into the headspace depends on the nature of the matrix and the strength of the matrix-analyte interactions. For thermally stable analytes, heating the sample is an efficient way to release analytes from the matrix to the headspace and improve sensitivity. Heating the sample provides energy for analyte molecules to overcome energy barriers which tie them to the matrix,[267] enhances the mass transfer process, and increases the vapour pressure of the analytes.[268] A small amount of water can also be added to soil samples to facilitate the release of analytes.[269] Heating solid samples helps to release analytes into the headspace therefore improving extraction efficiency. However, the absorption of analytes by the fibre coating is an exothermic process, which means that while the high temperature is good for the release of analytes from their matrix, it can adversely affect the absorption of analytes by the coating due to the decrease of the partition coefficients. Therefore, as the temperature increases the fibre begins to lose its ability to absorb analytes. Thus, an optimum temperature for SPME usually exists. If a sample can be heated to a high temperature whilst a low coating temperature is maintained then sensitivity will increase dramatically. This can be achieved by modifying the SPME device and using liquid CO_2 as a coolant.[270]

Accuracy and Precision SPME is primarily an equilibration technique and therefore calibration is necessary for quantitation. For relatively clean aqueous samples (<1 % organics) such as drinking water, external calibration normally works well and is carried out by spiking a known amount of target analytes into a representative matrix and then performing SPME in the same way as an unknown sample is analyzed. Analyte concentrations in unknown samples can then be determined by comparison of detector signals. Alternatively, for complex soil samples, external calibration may not work well because of matrix effects, and the method of standard additions may be more appropriate.[271,272] If analytes have very large partition coefficients, because of a strong affinity for the fibre coating or because of simultaneously heating the sample and cooling the fibre, exhaustive extraction can be achieved. In principle, calibration is no longer necessary but is usually carried out to confirm the extraction efficiency.

Precision in SPME should be very good because it is a single-step method and therefore random sources of error associated with multi-stage transfer of analytes are reduced. Typically, precision will be in the order of approximately 5 % relative standard deviation which may be further reduced when automated techniques are used.

Speed of Extraction In SPME, the rate at which analytes are extracted from a matrix is determined by the efficiency with which the sample is agitated. If a very effective agitation method is used (*i.e.* sonication) then equilibration times can be in the order of <1 minute.[265] For more practical agitation methods such as magnetic stirring, the equilibration time is usually between 2 and 60 minutes. The exact time is dependent on the agitation rate and the partition coefficient between the analytes and the fibre coating. If the SPME system is at equilibrium, then maximum sensitivity has been achieved. However, for practical purposes, the extraction time can be shortened and depends on the level of sensitivity required. In addition to the stirring rate and partition coefficient, the temperature of the sample also has an effect on extraction time because the mass transfer of analytes from the matrix to the coating is faster at higher temperatures.

Desorption

Unlike extraction which dictates the main experimental parameters in SPME, desorption is closely related to the efficiency of the chromatographic separation and the precision of quantitation and has an influence on the quality of data obtained from the SPME technique. Thermal desorption of analytes from the fibre coating is the most common technique and in most cases, the most effective. The thermal desorption is normally achieved by placing the fibre into the hot injection port of a gas chromatograph, whilst the carrier gas is flowing. As the temperature of the fibre increases, the coating-gas partition coefficient decreases and the ability of the coating to retain the analytes is quickly reduced. The constant flow of the carrier gas within the injection port also facilitates the removal of the target analytes. For volatile and some semi-volatile analytes, desorption takes place at a temperature range between 120 and 250 °C depending on the nature of the compounds. The analytes are removed from the fibre and are re-focused at the front of the gas chromatographic column by the temperature difference between the injection port and the column oven. The fibre coatings used have maximum temperatures at which they can be desorbed as excessive temperatures can cause the phase to bleed from the fibre. This temperature is dependent on the type of coating and the coating thickness and is obviously increased if the coating is chemically bonded to the fibre. Compounds with high

molecular weights (such as certain PAHs) may suffer from carry-over problems associated with too low a desorption temperature since most coatings cannot be heated above 300 °C.[271] When compounds cannot be desorbed from the fibre using high temperatures, desorption may also be accomplished by using an appropriate organic solvent.

2.3.3.2 Instrumentation

One of the main advantages of SPME is that is does not require expensive instrumentation or that existing instrumentation be excessively modified. The majority of research in the field of SPME has used "home-made" SPME devices constructed from normal GC microsyringes.[261,265,269,271,273,274,275,276] The syringes are used to protect the delicate fibre when it is initially introduced in the vessel containing the sample and whilst the fibre is inserted through the injection septa of the gas chromatograph for desorption and subsequent analysis. The metal plunger wire assembly in the microsyringe is removed and replaced with a fused silica optical fibre. The length of fibre required is experimentally determined and is dependent on its position in the injection port. Typically, conventional fused silica fibres have been used for the extraction of non-polar analytes and are coated with polyimide to increase the mechanical strength of the fibre. In order to use the fibre for SPME, the polyimide coating must first be removed by burning and then gently scraping off the charred section. After burning, the fibre becomes fragile and extra care must be taken when handling. Although the fibres are delicate, lifetimes of prepared fibres are in the order of 5-6 weeks with regular use.[261]

More recently, commercial SPME fibres have become available and are used in conjunction with a commercial fibre holder. The fibre holders are designed to be used either manually or by using a slightly modified configuration, for automated use. The fibres are supplied with a variety of coatings and coating thickness and are simply interchanged in the SPME holder when their lifetime is reached. The fibres can be used as received after a desorption cycle at the maximum desorption temperature that they will normally encounter in routine use. This eliminates the need for self-mounting and polyimide removal required when using "home-made" devices. The commercial fibres also have the advantage that fibre-to-fibre variation should be minimized.

Once the fibre and holder have been constructed, relatively minor alterations (if any) are required to a standard gas chromatograph in order to perform SPME. After the fibre has been inserted into a vial containing the sample of interest (either in the headspace or directly into the sample) and been allowed sufficient time to extract the target analytes, the fibre is withdrawn into the protective sheath of the syringe needle and inserted through a septum into a conventional GC injection port. The location of the fibre in the injection port is important as it affects the efficiency of the desorption and therefore the relative standard deviation and must be kept constant throughout analysis. Several injector designs have been utilized for fibre desorption including on-column[261] and conventional split-splitless.[265] However, by far the most popular design is that of the septum programmable injector (SPI) which has the advantage that it can be temperature programmed.[271,273,274,276] The other main modification which may be required when volatile organic compounds (VOCs) are to be extracted is the addition of oven cryogenics in order to efficiently focus the desorbed analytes at the front of the GC column and prevent peak broadening. If less volatile analytes are to be extracted then it is likely that they will be efficiently focused even at normal oven starting temperatures and cryo-cooling will not be necessary.

Practical application of any method to routine analysis requires that the method be automated. This is particularly important in environmental laboratories where sample throughput is high and sample turnaround time must be as short as possible. Automation of sample preparation is difficult for most sample types when established methods are used. Liquid-liquid partitioning, in particular, is almost impossible to fully automate and SPE usually requires robotic equipment to effectively remove the need for analyst time and is therefore quite expensive. With the advent of commercial fibres and holders, automated SPME has become available that requires only slight modification of a normal gas chromatographic autosampler.[277] Typically, modified SPME fibre holders are required that can be fitted to the GC autosampler carriage. SPME fibre holders may be obtained commercially which are specifically designed for use with GC autosamplers and that do not require further modification. Software changes are also necessary in order for the autosampler to perform the extraction and desorption stages in the analysis. Vials are then accurately filled with a known amount of sample (the vials must be filled to precisely the same height) and placed in the autosampler carousel to await analysis.

The advantages of automated SPME are similar to those of any automated technique. These include reduced manual sample handling and a reduction in sample to sample extraction and desorption variances which leads to improved accuracy and precision.

To date automated SPME does suffer from one disadvantage in that it is difficult to agitate the sample during the extraction stage because the fibre is situated in the GC autosampler. However, magnetic stirring has been achieved in automated SPME by removing the conventional syringe washing cup and replacing it with a micro-magnetic stirrer.[277]

2.3.3.3 Applications

Solid-phase microextraction is a recent technique that was first introduced in 1990. The majority of published research on the subject involves the removal of volatile organic compounds from aqueous matrices using non-polar fibre coatings. Typical analytes include substituted benzenes (benzene, toluene, ethylbenzene and xylene), or BTEX and are favoured because they are readily partitioned in the headspace above the sample and are easily desorbed at relatively low GC injector temperatures. The fibres used for the SPME are usually manufactured in the laboratory from commercial fused silica fibre and are used together with a "home-made" syringe assembly to protect the fibre. Conventional gas chromatographic instruments are used to desorb analytes from the fibres and for the subsequent analysis of the target analytes. For the analysis of BTEX compounds gas chromatography with flame ionization detection (FID) is often sufficient to obtain relatively low limits of detection (*ca.* 1 ppb) and the technique has been favourably compared with more conventional purge and trap methods as it offers great time savings.[273,276] Gas chromatography linked with ion trap mass spectrometry has also been successfully used to remove BTEX from aqueous samples and has the advantage of great sensitivity (pg levels) and a wide linear dynamic range (five orders of magnitude).[269,270,274] SPME has been used in an on-line configuration to analyze substituted benzenes (benzene, toluene and *p*-xylene) in flowing streams.[265] The technique is advantageous for the monitoring of contaminants in process streams and waste water effluents that are of particular interest to industry. The method used a conventional laboratory prepared fused silica optical fibre for the extraction. It was determined that in addition to convection introduced by flow in the system, an efficient means of agitation was required to achieve rapid extractions. Three agitation techniques; magnetic mixing, intrusive mixing and sonication were compared and it was found that sonication proved to be the most efficient means of agitation for sampling of flowing streams. Using this system, the substituted benzenes were found to equilibrate in less than 1 minute, which was close to the theoretical predictions assuming perfect agitation.

In addition to the more conventional GC detectors, Wittkamp and Tilotta used Raman spectroscopy to detect BTEX after extraction using SPME.[278] Raman spectroscopy is advantageous because it has the potential to be used as a remote field sensing device, removing the need to transport aqueous samples to the laboratory for analysis. The method used poly(dimethylsiloxane) "beads" that were capable of being quickly aligned in the Raman spectrometer as difficulty was encountered in aligning conventional SPME fibres. The time required for each BTEX compound to reach equilibrium between the solid phase and the aqueous phase was in the range between 16-30 minutes and afforded a pre-concentration enhancement of 2-3 orders of magnitude. The limit of detection using the most intense Raman bands was found to be 1-4 ppm with relative standard deviations of 3-9 %. No significant interferences were observed when "real" water samples, obtained from rivers and wells, were analyzed.

Other non-polar volatile organic compounds have been investigated using SPME. The technique has been used to determine volatile chlorinated hydrocarbons in air and water matrices which would commonly be analyzed by purge and trap methods.[279] The compounds studied include chloroform, carbon tetrachloride and tetrachloroethane which were extracted using a 95 μm poly(dimethylsiloxane) fused silica fibre and a "home-made" fibre holder. Extraction equilibration times varied from 1 minute (for the most volatile compounds in the gas phase) to 10 minutes (for less volatile compounds in the gas phase). For all compounds in the mixture (12) to be completely removed from the fibre, a 3 minute desorption at 200 °C was required as shorter desorption times caused carry-over of the less volatile compounds (carbon tetrachloride and tetrachloroethane). Cryofocusing of the analytes was required in order to ensure good peak shape. Electron capture detection was used to analyze the desorbed analytes and gave limits of detection of 1-130 ng l^{-1} for liquid samples. In the gaseous phase, the method had limits of detection in the parts per trillion (v/v) range when used with electron capture detection. A slightly wider linear dynamic range was observed for liquid samples (between 2-4 orders of magnitude) when compared to gas phase samples (2 orders of magnitude). In addition, liquid samples offered better precision (RSD of 1-5 %) when compared to gas phase samples (RSD between 1-7 %).

Non-polar fibre coatings have also been used to extract semi-volatile analytes from aqueous matrices. Poly(dimethylsiloxane) coated fibres (15 μm) were used to extract polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) from water samples.[280] Desorption and separation was performed on a bench-top gas

chromatograph-ion trap mass spectrometer fitted with SPI and oven cryogenics. Using the 15 μm fibre, equilibration time ranged from 6 to 60 minutes depending on the target analyte. Desorption was performed in the SPI which was held constant at 50 °C (3 minutes) before being ramped to 300 °C at 250 °C min^{-1}. The total desorption time for the fibres was 10 minutes. Detection limits ranging from 1 to 20 pg ml^{-1} were obtained for naphthalene, anthracene, benz[a]anthracene, benzo[a]pyrene, 2,2',5-trichlorobiphenyl and 2,2',3,4,5-pentachlorobiphenyl after only a 10 minutes sampling time with system linearity being established from low pg ml^{-1} levels to ng ml^{-1} levels for all compounds studied. The relative standard deviation obtained was comparable to the US EPA standard method and ranged from 10 % (for PAHs) to approximately 20 % (for PCBs).

A novel technique incorporating SPME as the extraction method has been shown to successfully analyze PAHs in complex samples. SPME with analysis by laser desorption ion trap mass spectrometry was used, where the silica fibre served as both the sampling medium and the sample support for laser desorption.[281]

Other non-polar semi-volatile compounds have also been shown to be successfully analyzed using fused silica fibres. Hexachlorocyclohexanes (HCHs) have been determined in soil samples using SPME incorporating a GC fitted with electron capture detection (GC-ECD) or mass spectrometer detection (GC-MS) for analyte desorption and analysis.[282] The study was carried out using a commercially available 100 μm poly(dimethylsiloxane) fused silica fibre which was placed in a soil solution. Equilibration was dependent upon the rate of agitation and was found to range from >60 minutes for an unstirred solution, to between 40-60 minutes for a magnetically stirred soil solution. Desorption was performed in a conventional split/splitless GC injector maintained at 220 °C. After optimization, a desorption time of 2.0 minutes was chosen. SPME was found to have detection limits in the range between 5 ng l^{-1} (using GC-ECD) and 80 ng l^{-1} (using GC-MS). The SPME-GC technique was then utilized in a study of the mobility of HCHs in wetland soils.

Although the majority of research in SPME has involved the extraction and analysis of non-polar organic compounds, the recent advent of more polar fibre coatings has allowed SPME to be used to study polar compounds.[283] Buchholz and Pawlsizyn investigated SPME as a technique to analyze phenols in aqueous samples using GC with FID and ion-trap MS detection.[284] Initial work was carried out using the more conventional non-polar poly(dimethylsiloxane) fibre coating. However, it was found that the coating was not capable of removing 2-chlorophenol, 4-nitrophenol or 2,4-

dinitrophenol which were not detected using either GC with FID or GC-MS. Also, the peak shape obtained for some of the compounds (in particular pentachlorophenol) was unsatisfactory. *In-situ* derivatization of the phenolic compounds (using acetic anhydride to convert the phenols to their corresponding acetates) prior to extraction using the non-polar coating enhanced both the overall peak shape and the recoveries of the majority of the phenols, excluding 2-nitrophenol. However, this additional analysis stage added time to the procedure and a polar fibre coating (poly[acrylate]) was investigated for its potential in extracting all of the phenols. The results obtained using this coating demonstrate that it was possible to extract all of the target phenols from water using a 40 minute equilibration time. The slower equilibration time with the poly(acrylate) coating compared to the poly(dimethylsiloxane) coating is due to the nature of the solid phase (poly[acrylate]) which does not allow the easy diffusion of analytes. Low pH levels and saturated salt conditions were found to significantly enhance the sensitivity of the method which reported a detection limit of sub parts per billion, and a precision of 5-12 % RSD.

To date, because SPME has typically been used to extract analytes from aqueous samples, the majority of publications on the subject have been concerned with environmental applications. However, SPME has occasionally been used in other analytical areas and in particular in food analysis. Polyimide-coated fused-silica fibres (having outer diameters of *ca.* 170 μm) have been used to extract caffeine from beverages.[285] The protective polyimide coating was first removed (burnt off) from the fibres and the exposed section dipped into the beverage (regular coffee, decaffeinated coffee and tea) for an exposure time of 5 minutes. No further sample preparation was necessary apart from the addition of a methanol solution containing ^{13}C labelled caffeine. After the extraction, the fibre was inserted into a heated split/splitless injection port (300 °C) to thermally desorb the analytes into the GC-MS system. Quantitative reproducabilities reported were *ca.* 5 % (RSD) and the entire scheme, including sample preparation and gas chromatographic analysis was completed in approximately 15 minutes.

SPME has also been utilized in the analysis of flavour components in a wide variety of foods including saffron, dill seed, spearmint gum and food packaging material.[286] A commercially available 100 μm poly(dimethylsiloxane) coated fused-silica fibre was used to sample the flavour components from the headspace of the various samples. Extraction times used varied from 1 to 15 minutes, with desorption times in the range between 5 seconds and 2 minutes (200-250 °C), depending on the sample. Compounds such as limonene and pinene were detected using FID detection after

separation on a chiral-phase capillary GC column. SPME is shown to be a valuable technique for the verification of flavour addition or the detection of off-flavour components and packaging-derived contaminants.

2.3.4 Microwave-Assisted Extraction

The extraction of semi-volatile organic compounds from environmental solid samples (soils and sediments) has been routinely performed by solvent extraction for many years. In particular Soxhlet extraction (discussed in section 2.2.2.1) is still the preferred choice for the majority of work carried out on contaminated solids in environmental laboratories. Unfortunately, Soxhlet extraction is time-consuming and generates large volumes of waste organic solvents. Additionally, the technique is difficult to automate and therefore different approaches to extract compounds of environmental concern from solid matrices are currently being evaluated. One of the more promising techniques to be assessed is the use of microwave energy to heat solid-solvent mixtures in closed vessels housed in a microwave oven. Microwave-assisted extraction is currently routinely used in inorganic analysis but also offers similar benefits to organic sample preparation requiring solvent extraction. These include a reduction in the amount of time required to perform the extraction when compared to Soxhlet and a substantial reduction in the amount of organic solvents used. In addition, the commercial microwave units now available are capable of simultaneously extracting multiple samples.

2.3.4.1 Theory[287]

Microwave energy is a non-ionizing radiation that causes molecular motion by migration of ions and rotation of dipoles, but does not cause changes in molecular structure. It has a frequency range from 300 to 300,000 MHz with four frequencies used for industrial and scientific purposes, the most common being 2450 MHz which is used in all domestic microwave units.

The way in which a sample is heated by microwave energy is dependent upon its dissipation factor. The dissipation factor is a ratio of the samples dielectric loss to its dielectric constant. Here the dielectric constant is a measure of the samples ability to obstruct microwave energy as it passes through while the dielectric loss is the amount of input microwave energy that is lost to the sample by being dissipated as heat.[288]

When microwave energy penetrates a sample, the energy is absorbed by the sample at a rate dependent upon the dissipation factor. Typically, energy is quickly absorbed and dissipated as microwaves pass into a sample and therefore the greater the dissipation factor of a sample, the less penetration of the microwave energy. Normally microwave energy is lost to the sample by two mechanisms, ionic conduction and dipole rotation, which may take place simultaneously. Ionic conduction is the conductive migration of dissolved ions in the applied electromagnetic field. This ionic migration is a flow of current which results in heat production due to resistance to ion flow. All ions in solution contribute to the conduction process, but the fraction of current carried by any given species is determined by its relative concentration and its mobility in the medium. Therefore, the losses due to ionic migration depend on the size, charge and conductivity of the dissolved ions.

The relative contribution of ionic conduction to heating in organic analysis using non-ionic solvents may be small in comparison to dipole rotation. Dipole rotation refers to the alignment, due to the electric field, of molecules in the solvent and sample that have permanent or induced dipole moments. As the field decreases, thermally induced disorder is restored which results in thermal energy being released. At 2450 MHz, the alignment of the molecules followed by their return to disorder occurs 4.9×10^9 times per second, which results in rapid heating. The polarizability of the solvent molecules obviously depends on the nature of the solvent and its dielectric constant. Therefore the greater the dielectric constant the more thermal energy released and the more rapid the heating for a given frequency. Non-polar solvents such as hexane and toluene with low dielectric constants are not affected by microwave energy and therefore require polar additives if they are to be used as solvents in microwave extraction. However, the efficiency of heating by dipole rotation depends upon the samples characteristic dielectric relaxation time (the time which the molecules delay in responding to the field change, or conversely, the time for them to revert to disorientation) that in turn depends upon the sample temperature and viscosity.[289] The maximum energy conversion per cycle (electromagnetic to heat) will occur when the angular frequency of the microwave energy (radians per second) equals the reciprocal of the dielectric relaxation time of the sample. If the two values are considerably different for a sample, the dissipation factor will be low resulting in poor heating efficiency.

A typical Soxhlet extraction by conductive heating will be completed in approximately 5-14 hours. Alternatively, closed vessel extractions by microwave heating can be completed in approximately 15 minutes. The difference is due to the sample heating method. In conductive heating, vaporization at the liquid surface

causes a thermal gradient to be established by convection currents and only a small portion of the liquid is at the temperature of the heat as applied to the outside of the vessel. Therefore this method of heating is quite inefficient. In comparison, microwaves heat all of the sample simultaneously without heating the vessel. Therefore, with microwave heating, the solution reaches its boiling point very rapidly. In addition, the Soxhlet extraction system uses relatively cold solvent since it is condensed in a reflux condenser before passing through the extraction thimble (discussed in greater detail in section 2.2.2.1). The extracting solvent therefore never reaches a temperature much above that of the condensing water used. Also, because in microwave extraction the solvent is in a sealed system, it is capable of reaching far greater boiling points than at atmospheric pressure. Polar solvents, such as, acetone and dichloromethane are heated to approximately 100 °C above their normal atmospheric boiling points.[290] It is these high extraction solvent temperatures combined with rapid heating which increases extraction efficiency and therefore greatly reduces extraction time with a microwave system.

2.3.4.2 Instrumentation

A typical microwave instrument used for heating analytical samples consists of six major components: the microwave generator (magnetron), a circulator, the wave guide, the microwave cavity, the mode stirrer and a turntable. In addition to the microwave unit requirements, specially designed sample vessels are also required. This list does not include any safety features or temperature and pressure monitoring devices. Microwave energy is produced by the magnetron, which is directed along the wave guide and projected into the microwave cavity where the mode stirrer distributes the incoming energy in many directions. The percentage of the incoming energy that is absorbed depends upon the sample size and dissipation factor. The six components are discussed in more detail below and shown schematically in figure 2.12.

The Magnetron[291]

The magnetron is a cylindrical diode with an anode and a cathode. A magnetic field is superimposed on the diode in alignment with the cathode. A potential difference of several thousand volts is reached across the diode and the released electrons, under the influence of the magnetic field, resonate. The anode is made from a ring of coupled resonant cavities which cause the magnetron to oscillate under the resonating electrons. The oscillating electrons give energy to the microwave field that radiates from an antenna enclosed in a vacuum tube that is situated in the wave guide. In a

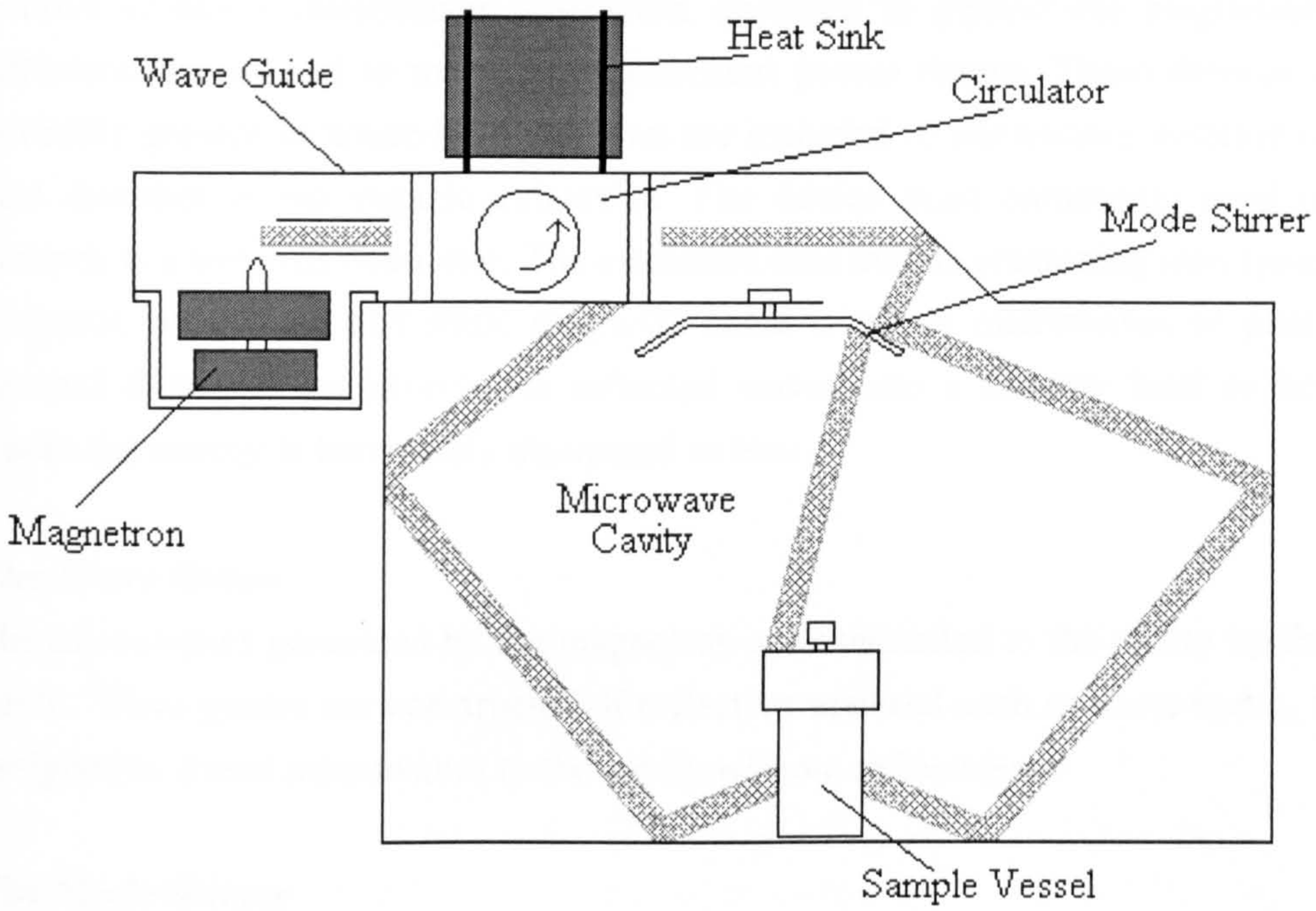


Figure 2.12 Schematic Representation of a Microwave Unit used in Sample Preparation.

fixed-tuned magnetron instrument, the oscillations are designed to release microwave energy at a certain frequency, commonly 2450 MHz. In these instruments, the magnetron converts approximately half the electrical input power into electromagnetic energy with the remaining energy converted to heat that is removed by air cooling.

In microwave systems used for sample preparation, the power output of the magnetron is controlled by "cycling" the magnetron to obtain an average power level. Thus, to obtain 50 % power from an instrument the magnetron would be switched on for half a set time period and then switched off for an equal half period. The length of the time period is dependent upon the application required from the instrument, but for analytical purposes is kept short (0.5 s) because heat losses can be significant during a long off period.

The Circulator

Microwave extractions usually involve relatively small volumes of solvents (typically between 30 and 60 ml) that do not have very high dissipation factors at 2450 MHz. Therefore, not all of the incident microwave energy is absorbed by the sample which results in reflected microwaves inside the cavity. The power output of a magnetron can be affected by overheating resulting from reflected microwaves. Devices that

remove reflected microwaves have been designed to protect the magnetron when reflection occurs and to maintain a consistent power output. These devices are not normally present in house-hold units but are included in microwave systems used for acid dissolution and organic extraction. The device most commonly used in these systems is a terminal circulator. The circulator uses metals containing iron (possessing magnetic properties) and static magnetic fields to allow microwaves to pass in the forward direction but diverts the reflected waves into a dummy load or heat sink where the energy is harmlessly dissipated as heat.

The Wave Guide

The microwaves generated by the magnetron are channelled to the cavity by the wave guide. Wave guides are constructed of reflective material such as sheet metal, and are designed to direct microwaves to the cavity without reflectance.

The Mode Stirrer

The mode stirrer is a fan-shaped blade that is used to reflect and mix the energy entering the microwave cavity from the wave guide. A mode stirrer assists in distributing the incoming energy so that the heating of the sample will be independent of position.

The Microwave Cavity

The microwave cavity is the area of the microwave unit where the microwaves are transmitted by the wave guide, and contains the sample vessel. Microwaves entering the cavity are repeatedly reflected from wall to wall in well-defined pathways that give reconizable patterns (or modes) having a beginning and an end. The microwaves entering the cavity intercept samples placed inside the cavity, and lose energy with each interaction until no energy remains in a given wave. When a sample has a low dissipation factor, the microwaves continue to be reflected, and have a greater chance of finding their way back to the magnetron. Microwave cavities are constructed from metal and therefore are usually coated with PTFE to prevent corrosion by solvent vapours which may escape from the sample vessel.

The Turntable

As mentioned in the previous paragraph, when microwaves enter the cavity, they are distributed in modes by reflection from the metallic surfaces of the cavity (reflection is still possible in the coated cavity because PTFE does not absorb microwave energy). When a single sample vessel is placed inside the cavity, the waves intercept the vessel and a percentage of energy in the wave is absorbed depending on the

sample itself. However, if two vessels are placed in the cavity, the vessel positions within the cavity determines the level of exposure of the vessels to the established microwave pattern. If one vessel has greater exposure to the established microwave pattern than the other, it will be heated differently and temperatures and pressures inside the vessel will be non-uniform. The uniformity of heating multiple vessels can be greatly increased by rotating the vessel on a turntable. Turntables are available to rotate 360° continuously, or that alternate back and forward 180°. The latter design is used when monitoring devices, such as pressure and temperature sensors are connected to the vessels.

Containers used for sample vessels are constructed from low-absorptive materials so that the microwaves are not absorbed by the vessel but will pass through the vessel to the sample / solvent mixture inside. Teflon PFA ([perfluoro alkoxy]ethylene) is the most commonly used material in the construction of microwave sample vessels although fused quartz and glass are also acceptable. Teflon PFA is widely used in all modern inorganic and organic microwave sample preparation applications but was initially used in acid dissolution because it is resistant to all acids and has a melting point of approximately 300 °C. Teflon PFA vessels may be used with conventional heating methods, but their use is limited because Teflon PFA is an extremely poor conductor of heat. However, this is an ideal situation for microwave heating since it is also transparent to microwaves and therefore allows the sample inside the vessel to be heated directly whilst the vessel walls act as an insulator.

2.3.4.3 Applications

Microwave heating has long been used for wet ashing procedures used in inorganic metal analysis. Standard techniques for wet ashing involve dissolution of the sample in a mineral acid for an extended period of time and it is well known that the acids used are capable of digesting materials more quickly at elevated temperatures and pressures.[292] Traditional heating was performed in chemically inert PTFE lined steel "bombs", in a conventional oven. These methods were time-consuming and were costly if the PTFE liners and steel jackets required replacing. These disadvantages prompted the investigation into microwave energy as an alternative heat source. The use of microwave energy to heat acids in wet ashing techniques was first demonstrated in 1975 using an open vessel design.[293] However, open-vessel work involves the risk of contamination as well as mechanical or volatile loss of analytes. In addition, open-vessel arrangements also limit the maximum sample temperature to

the boiling points of the acid used. These problems were overcome by using closed PTFE digestion vessels to obtain high temperatures and pressures needed for the digestion of difficult matrices such as steel[294] and geological samples,[295] including soils.[296]

Early research into microwave acid digestion used conventional household microwave ovens as a source of the microwave energy. The equipment required extensive modification in order to be used safely and it may be for this reason that microwave dissolution of inorganic samples has not completely superseded classical procedures. More recently there has been an increased impetus in the field of microwave sample preparation techniques because of the development of specially designed commercially available equipment. These microwave units address such inorganic analytical problems as the removal of acid fumes, sample power reflection and microwave field inhomogeneity.[297] The units are capable of accurate measurement of elevated dissolution temperature by the use of modern fibre optic thermometry[298] which has several advantages over conventional thermocouples that are difficult to construct in the narrow, shielded configurations required for acid dissolution in microwave systems. In addition to the developments in microwave units, until a strong, inert, microwave transparent container for acid dissolution was designed from Teflon PFA, closed vessel experimentation was dangerous and limited.[299]

The use of microwave energy to assist organic analysis was developed at a later stage than its inorganic equivalent. As in early inorganic analysis, house-hold microwave appliances were again used to generate the microwave energy which was used to heat organic solvents to extract analytes from solid samples.[300,301] The main difference in the instrumentation required for organic extraction compared to that for inorganic dissolution is in the safety requirements for working with organic solvents. The problems associated with working with flammable solvents in closed systems heated by microwaves was overcome in early work by only applying microwave energy in short time periods to the sample / solvent mixture. Using this technique, the solvent was never allowed to boil. More recently, commercial microwave instruments have become available which allow organic solvents to operate at temperatures well above their atmospheric boiling points. The pressure and temperature inside the Teflon PFA extraction vessel can be closely monitored by an in-line pressure transducer and fibre-optic thermometry, respectively. This enables either the extraction temperature or pressure to be set prior to extraction and electronically maintained at these set-points by automatically stopping and starting the magnetron. Several safety features are incorporated into the modern microwave extraction unit, specifically to allow the safe

usage of flammable and possibly explosive organic solvents. The extraction vessels used have an in-built rupture membrane which is rated to a fixed pressure. If the pressure inside the vessel exceeds the maximum operating pressure of the PTFE rupture membrane, the contents of the vessel are vented, *via* a PTFE tube, into a well or collection unit located in the centre of the microwave oven cavity. A solvent detection system which is situated in the exhaust duct of the system is capable of detecting small quantities of organic solvent vapour. When escaping solvent is detected the system automatically stops all microwave energy entering the cavity.

Although microwave-assisted extraction is a relatively new concept compared to its use in inorganic analysis, commercial microwave units based on the system described, have been used to remove analytes from solid samples. The method has recently be used to extract polyolefin additives from polymeric materials[302] and organochlorine pesticides from sediment.[303] However, although the second reference utilized a commercial microwave unit, the safety features mentioned above were not present and therefore the solvent / sample mixture was never allowed to boil. Microwave-assisted extraction has also been used to remove polycyclic aromatic hydrocarbons from soil reference samples where it compared favourably with standard techniques such as Soxhlet and sonication.[304,305] In these examples hexane / acetone solvent mixtures (1:1) were used to extract samples at various temperatures and for different time periods. Higher recoveries were found at increased temperatures although extending the extraction time had no significant effect on extraction efficiency. However, no attempt was made to assess the performance of different solvent ratios in the system.

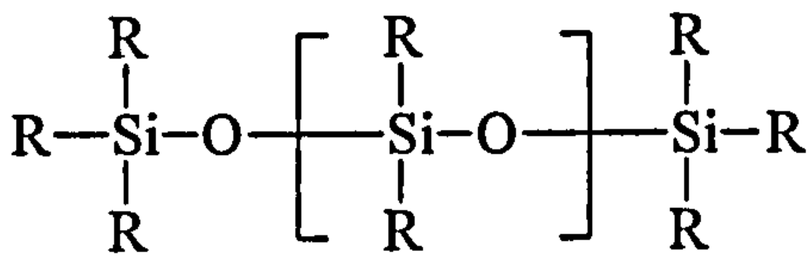
2.4 Chromatographic Organic Analysis Techniques

Chromatography is a technique in which the components of a mixture are separated based upon the rates at which they are carried through a stationary phase by a fluid mobile phase. Chromatography is widely used for the separation, identification, and determination of the chemical components in complex mixtures. No other separation method is as powerful and generally applicable as chromatography. In particular, in the field of environmental organic analysis, chromatography is an essential tool because of the very complex nature of the samples encountered and the chemical similarity of many of the analytes of interest. Chromatography has been used throughout the research as the analysis technique used to assess the performance of the sample preparative method being evaluated.

Chromatographic methods fall into three main categories based on the nature of the mobile phase. The three types of phases include gas, liquid, and supercritical fluids. Both gas and liquid chromatography have been used within the project.

2.4.1 Gas Chromatography

In gas chromatography (GC), an inert carrier gas serves as the mobile phase that elutes the components of a mixture from a column containing an immobilized stationary phase. Typical mobile phase gases include nitrogen, hydrogen, and argon, with helium being the most commonly used. Modern GC is almost always performed using fused silica capillary columns containing an immobilized liquid phase, as a stationary phase. Ideally the liquid phase should have properties which include; low volatility, thermal stability, and chemical inertness. The choice of stationary phase depends upon the type of analytes to be separated and in particular their polarity.[306] The majority of analytes analyzed by GC throughout the project are non-polar in nature and therefore are efficiently separated on non-polar column phases and in all cases, a general purpose non-polar stationary phase has been utilized. Typically, stationary phases are based on poly(dimethylsiloxane), similar to those used as SPME fibres, that have the general structure:



where R is a methyl group.

The stationary phase is usually chemically bonded to the silica surface of the column to reduce the occurrence of "bleeding" in which small amounts of immobilized liquid is carried out of the column during the elution process.

The GC used in the project is common with conventional systems, however, the principles behind the use of specific detectors to analyze different classes of pollutant is worthy of discussion.

The choice of the detection system in GC used to determine the concentration of analytes eluting in the gas stream is of great importance if efficient analysis is to be performed. All detectors should respond rapidly to minute concentrations of solutes as they exit the column, as the solute concentration in the carrier gas at any instant is

often less than parts per million. Additionally, the time required for a peak to pass the detector is typically less than one second; thus, the device must be capable of exhibiting its full response during a brief time period.

There are many detectors available for use with GC, often specifically designed to respond to certain classes of compounds. Three different GC detectors have been used in the research whose operation is discussed in greater detail below.

Electron Capture Detector (ECD)

The ECD is the most sensitive detector available for the non-destructive detection of electrophilic substances, such as chlorinated hydrocarbons, and is therefore particularly useful for pesticide residue determinations.[307] In the ECD, the effluent from the column is passed over a beta-emitter (a radioactive source that emits beta-particles) such as ^{63}Ni, absorbed on a platinum or titanium foil. An electron from the source causes ionization of the carrier gas (often nitrogen) and production of a burst of electrons which travel to the collector anode assembly under the influence of a pulsed polarizing voltage applied between the source and collector. The pulse frequency is varied to maintain a constant average current and if not used, the detector response would be non-linear. In the absence of an organic species, a constant standing current between the pair of electrodes results from this ionization. However, the current decreases in the presence of organic molecules that tend to absorb electrons, forming ions which travel much more slowly than electrons. Therefore as analytes are eluted from the column into the detector, the reduction in the current between the foil and the collector can be used to detect and quantify the electrophilic compounds (the reduction in current is proportional to the amount of analyte present). If the ECD is used in conjunction with capillary GC, a make-up gas (nitrogen) is required to efficiently sweep the eluants through the detector since the internal volume of the ECD is relatively large. The ECD is routinely operated at temperatures below 400 °C, although the temperature should be high enough to ensure no analytes are condensed on the foil or collector plates. However, the higher the temperature, the greater the migration rate of the ^{63}Ni into the backing material. This process reduces the electron flux and the life expectancy of the foil.

The ECD is selective in its response and highly sensitive to electronegative functional groups, such as halogens, peroxides, and nitro groups. It is insensitive to compounds such as alcohols and hydrocarbons. The high selectivity of the response towards halogenated compounds dictates that chlorinated solvents must not be used with GC-ECD. Typically, if dichloromethane has been used as an extraction solvent during

sample preparation, the solvent is evaporated and solvent exchanged for a compatible solvent such as *n*-hexane which shows little detector response.

It is this high sensitivity, combined with selectivity towards halogenated molecules which was utilized in the project to successfully determine extracts containing chlorinated pesticides.

Nitrogen / Phosphorus Detector (NPD)

The NPD is a thermionic detector that is sensitive to organically-bound nitrogen and phosphorus.[308] It can be operated in one of two modes:

- NP mode in which both nitrogen and phosphorus are detected and quantified
- P mode in which phosphorus only is detected and quantified

NP Mode of Operation

Principles of Operation In the NP mode of operation, carrier gas and sample are mixed with hydrogen and impinge upon an electrically heated, negatively charged rubidium glass bead. A low concentration of hydrogen is maintained to favour pyrolysis of the sample and the formation of stable intermediate products of nitrogen and phosphorus. Excited rubidium atoms are released from the surface of the hot bead and react with intermediate products to produce rubidium cations (Rb^+) and anions of nitrogen and phosphorus. The rubidium cations return to the bead and the anions migrate to the collector electrode where they are detected and the resulting current is amplified. An example of the reaction scheme is shown below in figure 2.13.

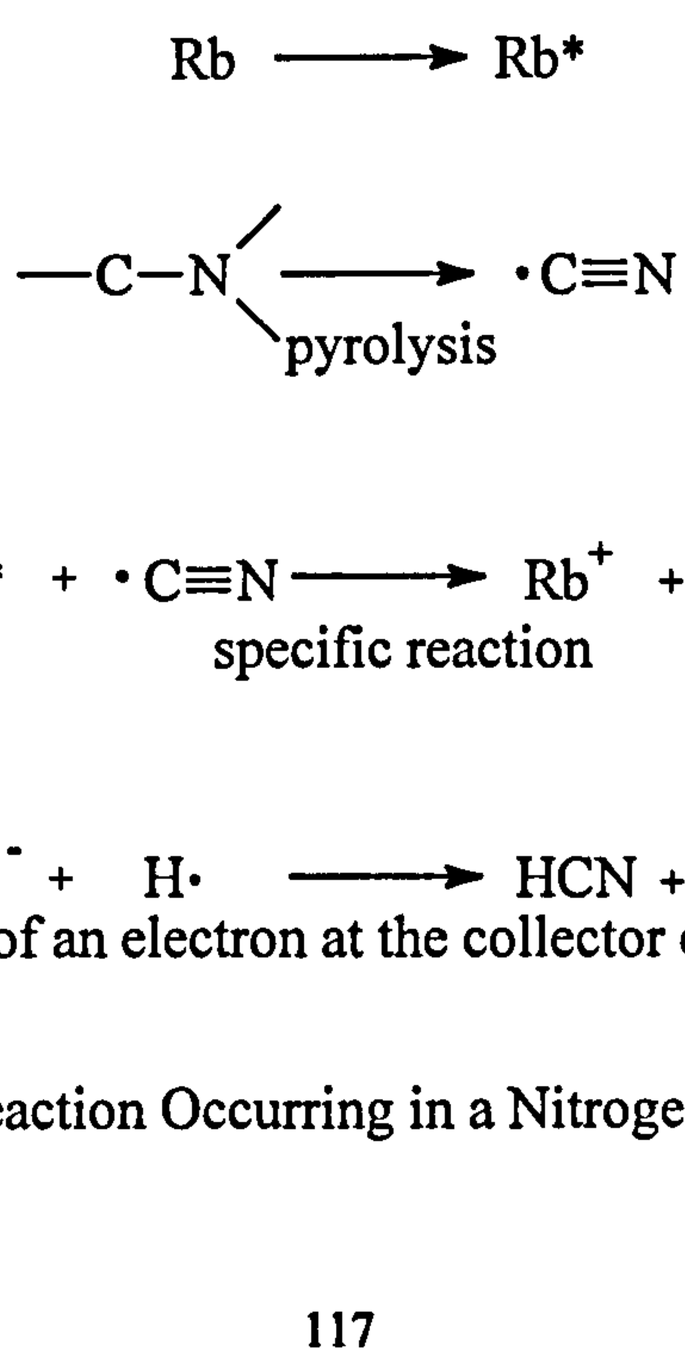


Figure 2.13 Example of a Reaction Occurring in a Nitrogen / Phosphorus Detector.

Operating Procedure NPD operation requires hydrogen, air, and either nitrogen or helium as a carrier and/or make-up gas. The amount of hydrogen relative to the other gases will affect the nitrogen-to-carbon and phosphorus-to-carbon specificity of the detector. The nitrogen-to-phosphorus specificity may also vary somewhat but the detector cannot be tuned to respond to only nitrogen or only phosphorus. Typical gas values (depending on detector manufacturer) are:

Air	175 ml min^{-1}
Hydrogen	5 ml min^{-1}
Carrier gas	30 ml min^{-1}*

* For capillary columns carrier gas + make-up.

Hydrogen Optimization The selectivity of the NPD is dependent upon the hydrogen flow and although typical values of 5 ml min^{-1} are recommended, the optimum flow to maximize the nitrogen-to-carbon selectivity must be determined for each individual bead. A test sample, containing compounds with both nitrogen and phosphorus atoms, as well as a normal hydrocarbon, is introduced into the system and the response to nitrogen *versus* hydrocarbon molecules is evaluated with the hydrogen flow adjusted between 4-6 ml min^{-1} to optimize this. Once the hydrogen flow is set it does not require altering for the remaining lifetime of the bead.

P Mode of Operation

Principles of Operation In the P mode, carrier gas and sample are mixed with a large volume of hydrogen that is ignited to produce a hot flame, which impinges on a negatively charged rubidium glass bead. Conditions are such that it is not necessary to heat the bead electrically and the stable intermediate nitrogen species of the NP mode are not produced. Combustion products of phosphorus react with the rubidium bead in the same manner as in the NP mode to produce anions, which are collected and the resulting current amplified. Any anions produced in the flame from the combustion products of organic molecules (flame ionization detector response) are removed from the flame by an electrically grounded detector jet.

Operating Procedure Typical flow-rates for P mode of operation are:

Air	280 ml min^{-1}
Hydrogen	40 ml min^{-1}*
Carrier gas	30 ml min^{-1}**

* With hydrogen as the carrier gas, the flow-rate of the carrier gas should be subtracted from the flow-rate for hydrogen above.

** For capillary columns carrier gas + make-up.

Bead Performance In either mode of operation, the sensitivity of the detector depends greatly on the bead temperature and therefore the bead current. Each bead has a particular current (*i.e.* bead surface temperature) at which the gas phase chemistry will initiate. In order to operate the detector it is necessary to allow the bead to reach this ignition point in a controlled manner. This is usually achieved (with all temperature zones and gas flows on) by gradually increasing the bead current from a low starting point in small increments (0.1 A) and allowing the bead to equilibrate after each current change. Eventually, an increase will cause the NPD baseline voltage to increase dramatically indicating that the bead has reached its ignition point. Increasing the bead current increases both the signal and noise produced from the detector, which in turn makes the detector slightly more sensitive. However, in order to prolong the life of the bead, the current is normally run at the lowest setting that will achieve acceptable performance.

As with ECD, chlorinated solvents must not be used with the NPD. These solvents generally cause an abrupt increase in both detector background signal and sample response. The effects appear to be associated with a temporary adsorption of chlorinated species onto the surface of the hot alkali-ceramic bead. Also, in general, any halogenated compounds will degrade bead performance, so stationary phases containing these compounds should be avoided as well as mixtures containing appreciable concentrations of chlorinated analytes. Once again, if chlorinated solvents have been used in sample preparative methods, they must be switched for an acceptable solvent such as *n*-hexane. In addition to problems with chlorinated solvents, the NPD is also sensitive to silyl derivatizing agents and column bleed, both of which reduce the lifetime of the bead.

Mass Selective Detection (MSD)

A mass spectrometer is a powerful detector for a chromatograph as not only is the usual chromatographic data obtained (quantitative information), but also mass spectral information for every point in the chromatogram. All mass spectrometers are based on the principle that the path of an individual molecule that is electrically charged (ionized) can be controlled in a mass dependent way by electric and/or magnetic fields, but there are several ways to ionize the molecules and to analyze the ion beams that are produced.[309]

Several different types of mass spectrometer are routinely coupled to GC including high resolution mass spectrometers, quadrupole mass filters and ion-trap mass

spectrometers. However, by far the most common is the quadrupole arrangement which has been exclusively used throughout the research.

Ionization Method Electron ionization (EI) is the basic technique upon which qualitative mass spectral analysis has been developed. In EI, electrons are emitted for a filament (tungsten or rhenium) by applying an ac voltage which restively heats the metal. In addition to the ac voltage, the filament also carries a -70 V dc bias voltage. The filament is conventionally housed outside and immediately adjacent to a hole in the ion source chamber, with the whole arrangement (together with the mass analyzer and ion detector) being sealed in a vacuum (approximately 10^{-5} to 10^{-7} torr) obtained using a diffusion pump in conjunction with a turbomolecular pump. Electrons emitted from the filament enter the ion source through the small window and are attracted by an electron trap, or collector, held at a potential that is positive with respect to the filament (*i.e.* 70 V). A small permanent magnet, together with a series of electrical lens plates are used to focus the electron beam which collides with the neutral molecules that enter as effluent from the GC column. Positive ions are formed when a neutral molecule collides with the electron beam in such a manner that a non-bonding, or lowest energy, orbital electron is displaced from the neutral molecule to form a radical ion. The excess energy transferred from the high-energy ionizing electron beam to the newly formed ion can be dissipated only by resonance stabilization or fragmentation. EI mass spectra therefore contain a series of ions that are representative of a compounds ability to stabilize a positive charge. Multiple fragmentation paths are possible, even for simple molecules. The fragmentation patterns are specific and unique with regard to molecular structure and allow a "fingerprint" of a particular molecule to be derived. The molecular fingerprint can be manually interpreted or be compared against reference spectra obtained under similar operating conditions.

Other types of ionization exist with perhaps the most common being chemical ionization (CI), which is a lower energy process than EI, and is more likely to yield mass spectra containing ions from which the *m/z* value of the molecular ion can be deduced. The conditions used to produce CI mass spectra involve the introduction of a so-called reactant gas (*e.g.* methane, iso-butane, or ammonia) into the ion source, from which ionic species are formed by the electron beam. These reactant ions then interact with molecules of the compound of interest. The conditions leading to optimal production of molecular ions are compound dependent, and CI is a much less robust technique than EI. However if no molecular ion is detected using EI, it may be possible to determine the molecular weight of a compound of interest using CI, or if

there is doubt as to whether an ion detected by EI is a molecular ion, then CI can be used for confirmatory purposes.

Other ionization techniques, such as fast atom bombardment and field desorption are not widely available, or are only applicable to specific types of compounds. They may however be of use in confirming an unknown compound when both EI and CI have failed.

Quadrupole Mass Analyzer Ion beams may be separated by a variety of electronic and magnetic principles with separation (in GC) predominated by quadrupole mass filters. The filter separates ions by accelerating the ion beam, produced in the ion source, with a low energy (5-10 V) into a region of superimposed direct and alternating currents defined by four metallic rods (quadrupoles). The rod potentials are paired in such a way that an ion is alternatively attracted toward a rod of opposite potential, and then repulsed when alternating current of radiofrequency (rf) energy inverts the field. For a fixed rf/dc potential, an ion of only a certain *mass-to-charge ratio* (*m/z*) will oscillate in a stable trajectory in the field defined by the potentials on the four rods. The momentum imparted to the ion by the accelerating potential in the ionization chamber, gives the ion beam an oscillating spiral path through the quadrupole rod assembly. By changing the rf/dc field strengths simultaneously, ions of varying *m/z* are filtered (*i.e.* only ions of stable trajectory are passed through the filter). During scanning, the rf/dc ratio is fixed because it defines the window of allowed *m/z* which is selected, thereby determining resolution between adjacent *m/z* values. Therefore a scan from *m/z* 35 to 550 amu can be achieved by ramping the rf/dc potentials in a rapid and repeated way, producing the impression of continuous and dynamic monitoring of a wide mass range.

Quadrupole mass filters have several advantages which make them compatible as a GC detector. The low voltages used in quadrupole ionization sources to accelerate ions out of the ion source and lens potentials below 100 V to focus them into the rod assembly make it relatively simple to connect GC inlet lines to the ion source without electrical grounding problems. The low energies used for ion acceleration are necessary to allow ions to drift through the mass filter at speeds slow enough to allow mass separation. Quadrupole mass filters are generally very compact, and rod lengths of approximately 15 cm can be accommodated in relatively small vacuum assemblies. Quadrupole instruments for EI are often built with a single diffusion pump that evacuates both the ion source and analyzer regions.

Ion Detection Regardless of how ionization and mass separation are accomplished, the mass differentiated ions then impact on one of two basic varieties of electron multiplier (discrete dynode or continuous dynode), which are related in principle to the more familiar photomultiplers of optical instrumentation. When ions strike a suitable (copper-beryllium or semi-conducting glass) surface with sufficient energy (1,000-2,000 V), the surface emits secondary electrons, which can be cascaded to produce a measurable electron pulse or current. In the discrete dynode electron multiplier, the mass-separated positive ion beam is attracted to the first surface (dynode), which is maintained at approximately -2,000 to -3,000 V. Secondary electrons emitted from the surface as a result of this collision, cascade to the next dynode, which is 100-200 V more positive than the first, giving rise to additional electrons, and the cascade continues along the 12-16 element dynode chain to ground potential where it is detected as a pulse of current with an amplification gain of up to 10^6 over that attainable by direct ion detection. Continuous dynode multipliers are a solid-state version of the discrete dynode with similar amplification, but in which the electron cascade proceeds along a single piece of semi-conducting glass tubing.

Recording of the Mass Spectrum In modern GC-MS, mass spectral information obtained from a quadrupole mass analyzer coupled to a gas chromatograph is recorded by computer data-systems in one of two modes:

Scan Mode The intensity of a mass spectrum is directly proportional to the amount of analyte present in the ionization chamber. In scan mode (or total ion current profile) the quadrupole mass filter is pre-set to scan a range of mass units in commonly less than a second. By assigning an intensity value to each recorded mass spectrum, a plot of relative intensity *versus* scan number (equivalent to retention time) can be made. In this way, mass spectral data obtained during a 30-45 minute gas chromatographic analysis can generate a file containing hundreds of mass spectra, each containing ion intensity data. The plot obtained resembles a common chromatogram from a conventional GC detector and can be used to quantitate the sample.

Selected Ion Monitoring In selected ion monitoring (SIM), a pre-selected number of ions known to be abundant in the analytes of interest, are scanned by the quadrupole mass filter instead of the entire mass range. These ions can be continually scanned throughout the chromatographic run or be assigned to retention time windows where only one or two specific ions are looked for over a particular time period known to coincide with target peak elution from the column. Although the nature of SIM does not allow the mass spectra it produces to be interpreted and requires additional

information about the sample, it does have advantages over total ion profiles. SIM is usually a more sensitive technique since only ions of interest are detected in a given scan. Additionally, all other interfering background ions are not seen in the resulting profile, greatly reducing the amount of noise in the chromatogram. SIM is therefore used with samples which are known to contain specific analytes and where sensitivity may be a problem.

2.4.2 High Performance Liquid Chromatography

In general, reverse-phase high performance liquid chromatography (RP-HPLC) is used for the separation of thermally labile or non-volatile compounds that cannot be separated using GC. Many of the principles behind (HPLC) have already be discussed in the solid-phase extraction theory section (2.3.2.1). These include the chemical nature of the stationary phases encountered (C_{18} being the most common) and the retention mechanisms behind analyte separation from the aqueous phase. In addition, the basic instrumentation required for HPLC is almost identical to that used for SFE (pumps and oven) with the exception of the detector which is seldom used in off-line SFE. In HPLC, relatively short columns (≤ 25 cm) packed with porous silica particles, with spherical or irregular shape, and nominal diameters of 10, 5, or 3 μm onto which are bonded different chemical functionality, are used to separate a range of analytes depending on their polarity.[310] Typical mobile phases in reverse-phase HPLC are mixtures of water (or buffers), methanol and acetonitrile which are pumped down the column at flow-rates between 1-3 ml min^{-1}. If the composition of the mobile phase is constant, the method is called "isocratic" elution. Alternatively, the composition of the mobile phase can be made to change in a predetermined way during the separation, which is a technique called "gradient" elution. Gradient elution is used in situations similar to those requiring temperature programming in GC, and is necessary when the range of retention times of solutes on the column is so large that they cannot be eluted in a reasonable time using a single solvent. Generally, for non-polar analytes, an increase in the amount of water present in the mobile phase increases the interaction of the analytes with the non-polar stationary phase and therefore the retention time. Conversely, increasing the percentage of organic solvent in the mobile phase has the opposite effect and elutes the analytes more quickly from the column. By altering the overall composition of the mobile phase (either isocratically or by gradient elution), a separation of analytes based on their relative interaction with stationary and mobile phases can be achieved.

There are several different detectors routinely used to detect analytes present in the eluant from an HPLC system, with by far the most common being an ultra violet-visible detector (UV-vis). In the UV -vis detector, the mobile phase from the column is passed through a small flow-cell (typically 1-8 μl volume) held in a radiation beam of a UV-vis spectrophotometer. These detectors are selective in the sense that they will only detect those solutes that absorb UV (or visible) radiation. Such solutes include alkenes, aromatics and compounds having multiple bonds between carbon and oxygen, nitrogen or sulphur. Conversely, the mobile phase used should absorb little or no radiation. Both fixed and variable UV-vis detectors are available. The variable types use a deuterium and/or tungsten filament lamp as the radiation source, and can operate between about 190 and 700 nm. Fixed wavelength detectors normally operate at 254 or 280 nm, but other wavelengths are possible. Polychromatic radiation is passed through the sample and is then focused on to the entrance slit of a monochromator, which passes a narrow band of wavelengths to the detector. The absorbance of the sample is found by comparing the intensity of radiation reaching the detector without the sample (blank) and after passing through the sample. To measure the absorbance at different wavelengths, the wavelength is changed by slowly rotating a diffraction grating in the monochromator. The radiation not absorbed by the sample then falls on a photomultiplier which converts the radiation into an electrical response in a similar way to the electron multiplier discussed in the previous section.

Chapter 3

Chemometrics

3.1 The Role of Chemometrics in the Investigation and Optimization of Operating Parameters used in Sample Preparation

Scientists, throughout the world, run experiments. The classical experimental approach is to study each experimental variable separately as this one-variable-at-a-time strategy is easy to handle and understand. Statistically speaking, each variable is called a factor and the effect they have on a particular system can be assessed by the response (y), which is some function of the factor(s) (x). For example, if the affect three variables have on the response is to be studied by the classical approach, the levels of all the variables except one are held constant whilst the remaining factor is changed. This experimental technique is therefore said to be univariate. However, this approach is not the most efficient way to study an experimental system. Two main problems arise from the use of this strategy:

1. If there are many different factors to study in a system this will represent an enormous number of experiments.
2. Only one factor is being varied at any one time, therefore the classical approach does not allow any interaction between factors to be estimated.

The first people to recognize these problems were agronomists and statisticians working at the beginning of the century.[311,312] Their studies usually involved a large number of parameters with each experiment lasting a long time. It was therefore pertinent for them to propose methods for organizing trials so that a combination of factors could be studied simultaneously. Statistics were used to initially design the experiments to ensure the maximum amount of information could be obtained whilst keeping the number of experiments low, and then to simplify the multivariate responses obtained. This early experimental design methodology was often highly theoretical and involved complex calculations and only in more recent years, with the advent of statistical packages on personal computers has experimental design become more widely accepted.

Experimental design methodology when used to study chemical systems has recently been termed chemometrics and offers several advantages including, fewer trials enabling a large number of factors to be studied, detection of interaction between factors, optimization of results and model-building from results. The term experimental design is usually used to describe the stages of (a) identifying the factors which may affect the result of an experiment, (b) designing the experiment so the

effects of uncontrolled factors are minimized, and (c) using statistical analysis to separate the effects of the various factors involved.[313]

Within chemistry it is normal to want to investigate a system by experimentation in order to obtain a greater understanding of the system with the hope of eventually optimizing it. Response surface methodology is an area of experimental design which deals with the optimization and understanding of system performance.[314] A response surface is simply defined as a graph of a system response against one or more of the system factors. In its simplest form, the response surface for one factor can be plotted in two-dimensions with response as the y-axis and the factor as the x-axis. With a multi-factor system, only two factors can be visualized at any one time in three-dimensional space, taking the form of a plot in x, y, z co-ordinates. Commonly all of the points on a response surface will not be known, instead, only a few values will have been experimentally obtained and the information will give an incomplete picture of the response surface. Common practice is then to assume a functional relationship between the response and the factor (that is to assume a model) and find the values of the model parameters that fit the data. Equation 3.1 shows the general form of the assumption.[315]

$$y = f(x_1, x_2, x_3, \ldots, x_n, \ldots) \qquad (3.1)$$

It is the role of the experimental design to ensure that the experiments undertaken can adequately describe the response surface for the factor space chosen (the limits of the factors) and can be used to fit (and diagnose) the model.[316]

3.1.1 Full Factorial Designs

Factorial designs are a very popular class of experimental design that are often used to investigate multifactor response surfaces. The word "factorial" does not have its usual mathematical meaning but indicates that many factors are varied simultaneously in a systematic way.[317] One of the major advantages of factorial designs is that they can be used to reveal the existence of factor interaction when present in a system. Important descriptors of factorial designs are the number of factors involved in the design and the number of levels of each factor. For example, if a factorial design has three levels (low, middle and high) of each of two factors (x_1 and x_2), it is said to be a 3 x 3 or 3^2 design. All of the possible combinations of the chosen factor levels are

present in the experimental design therefore, the description of a factorial design gives the number of factor combinations (*f*) contained in the design: $3^2 = 9$.

Two-level factorial designs are the simplest, but are widely used because they can be applied in many situations. The design can be notated as 2^2 and therefore consists of four separate experiments at different combinations of the two chosen factors. This arrangement is best illustrated using a simple graph showing the various factor combinations (figure 3.1).

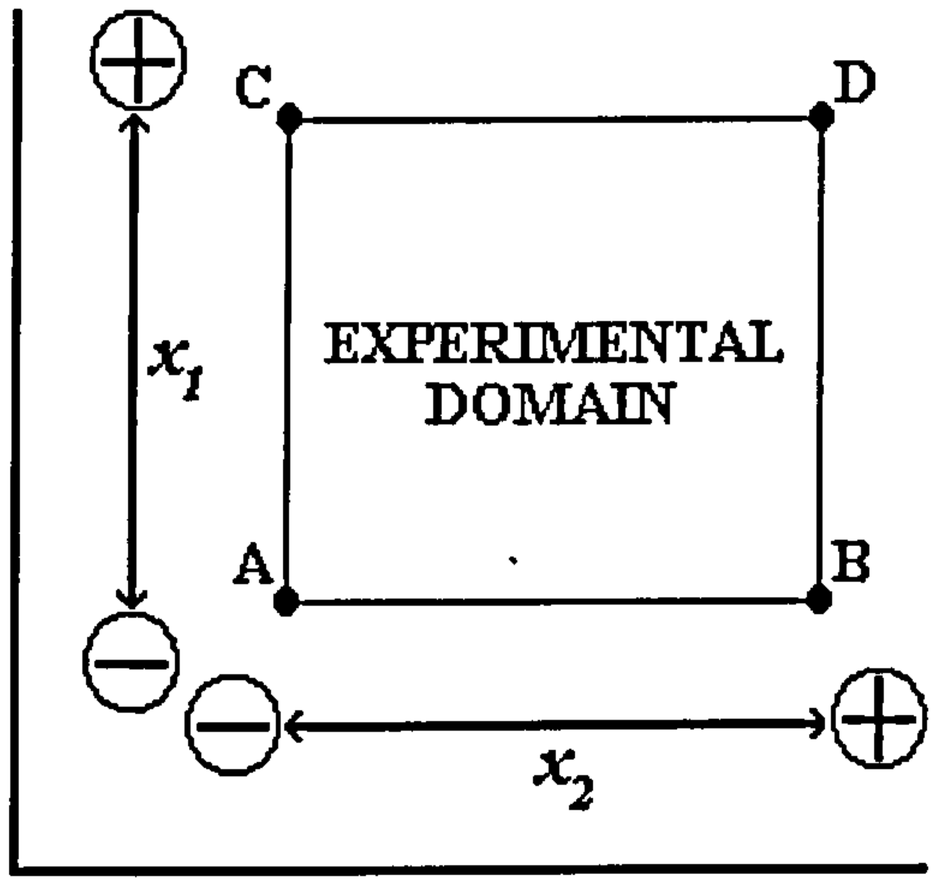


Figure 3.1 Representation of a Two-level, Two-Factor (2^2) Factorial Design.

The value of the levels chosen for a design (black dots labelled: A, B, C, and D) are dependent on the experimenter but must represent the experimental domain which is of interest. Limits of the levels may be set by instrumental constraints or by the chemical system which is being studied. Calculations for factorial designs are often greatly simplified if coding of factor levels is employed. It is common to code the lowest level as -1, the middle level (if present) as 0 and the highest level as +1. The coding of levels is achieved through equation 3.2.[318]

$$x^* = (x - c) / d \qquad (3.2)$$

where, x^* is the coded level, x is the actual value of the level, c is the design centre point and d is the distance between centre points and ± 1 values.

The experiments in the design generate four responses which are used to calculate the main effects of the factors in the experiment. The global effect of x_1 (similarly for x_2) is defined as half the difference between the average of the responses at the high level

and the average of the responses on the low level.[319] Also the interaction between x_1 and x_2 is defined as half the difference between the effects of x_2 at the high and low x_1 levels. This result applies whether the x_1x_2 or the x_2x_1 interaction is calculated. The magnitude and sign of the main and interaction effects then dictate the size of the effect each factor has on the experimental response (a positive sign indicates that the factor increases the overall design response and vice versa).

A more complex factorial design which is used to study three separate factors is the 2^3 design. The experimental domain is a cube and the eight experimental points chosen are the corners of the cube. This is represented in coded form in figure 3.2.

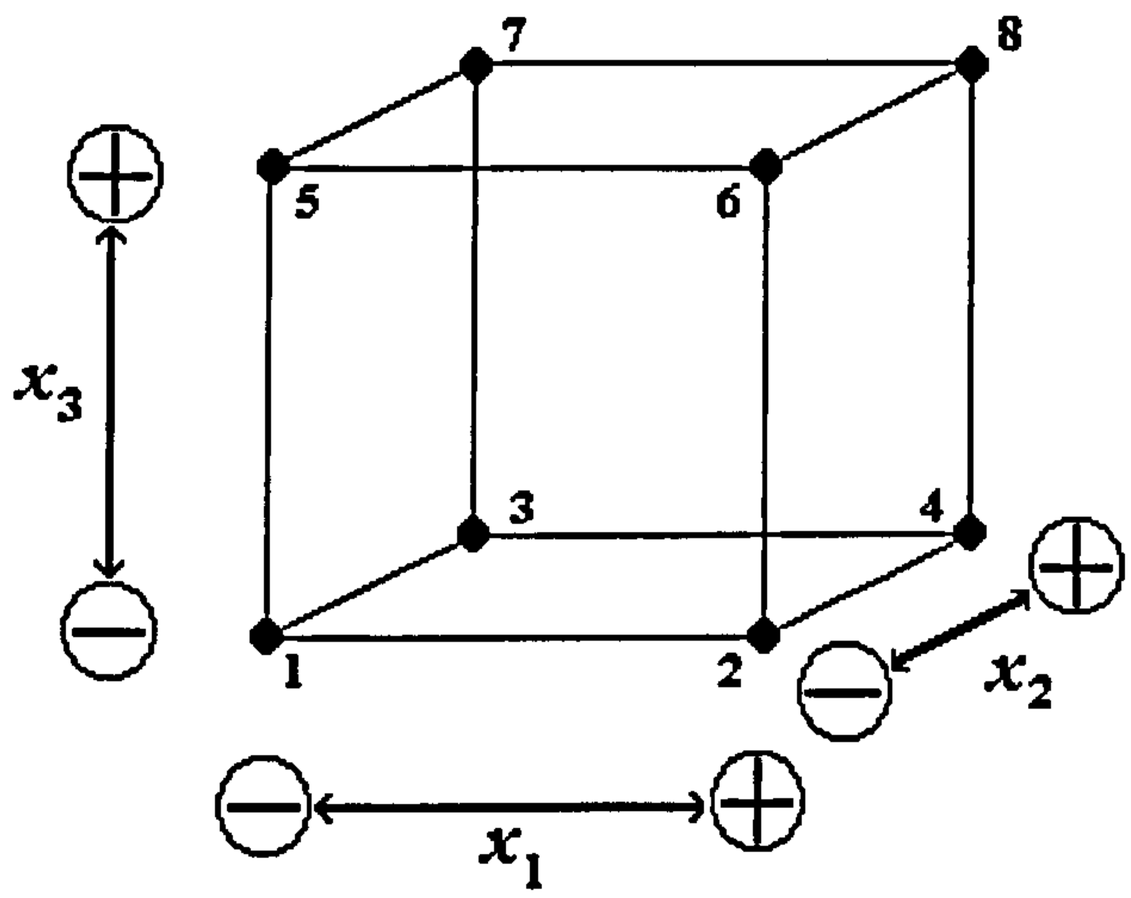


Figure 3.2 Distribution of Experimental Points Within the Experimental Domain of a 2^3 Design.

In this diagram, the plus and minus signs indicate low and high levels of each factor. The experiment can be written in the form of a design matrix (table 3.1) which illustrates all of the different factor combinations (2 x 2 x 2 = 8 in total). Factorial designs, like the ones above, are particularly useful for estimating the main effects of several factors and any interactions between them. With higher designs the classical approach, where the average of the responses at low and high levels is used to calculate the main and interaction effects, becomes time consuming and is statistically difficult to interpret. A linear model is most commonly fitted to the data from factorial designs and is of the form:

$$y_{1i} = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + \beta_{12} x_{1i} x_{2i} \qquad (3.3)$$

Experiment Number	Factor 1	Factor 2	Factor 3
1	-	-	-
2	+	-	-
3	-	+	-
4	+	+	-
5	-	-	+
6	+	-	+
7	-	+	+
8	+	+	+

Table 3.1 Design Matrix for a 2^3 Experimental Design (the 1 has been omitted from the levels for simplification).

which is of the same form as the general equation (3.1). This model gives estimates of an intercept term (β_0), a first-order effect (β_1), of the first-factor x_1, a first-order effect (β_2) of the second factor x_2 and a second-order interaction effect (β_{12}) between the two factors. This general equation can be fitted to the data using least squares regression analysis which estimates the model coefficients. The coefficients are calculated so that the sum of squares of the differences between the observations (y_i) and the fitted response ($\hat{y}_i$) is a minimum.[320] Once the model has been fitted to the data from the design, an estimate of how well the equation fits the data must be investigated. The errors associated with the model are assumed to have mean zero and unknown variance (σ^2). Also, the errors are assumed to be uncorrelated (the value of one error does not depend on the value of any other error).

In addition to estimating the coefficients, an estimate of the variance is required to test the significance of the factors in the model. Ideally, this estimate does not depend on the adequacy of the fitted model and this is only possible with several observations of response for at least one value of x. These repeat observations are frequently carried out at the design centre and are an evaluation of the pure error associated with the experiment.

A test for the "lack of fit" of the model is usually undertaken assuming that the sum of squares of the residuals (y_i - $\hat{y}_i$) is made from both the sum of squares of the pure error (obtained from replicates usually at the design centre) and the lack of fit. By simple subtraction, the sum of squares for the lack of fit can be calculated (equation 3.4) and hence a "mean square" (the mean square is equivalent to the variance), which takes into account the number of degrees of freedom in the system, determined.

$$SS_{LOF} = SS_{RE} - SS_{PE} \qquad (3.4)$$

where, SS_{LOF} is the sum of squares of the lack of fit, SS_{RE} is the sum of squares of the residuals and SS_{PE} is the sum of squares of the pure error.

The mean square of the lack of fit can then be compared with that obtained from the pure error and used to statistically test whether the model exhibits any significant lack of fit to the experimental data. In this case the F-ratio obtained from division of the mean square of the lack of fit by the mean square of the pure error can be compared to F-distribution tables at the required confidence limit to decide if the model shows significant lack of fit.

Analysis of variance, frequently abbreviated to ANOVA (ANalysis Of VAriance), is a powerful statistical technique which can be used to estimate and separate the different causes of variation.[313] ANOVA can be used to separate any variation which is caused by changing the controlled factor, from the variation due to random error. This is therefore a statistical test to see whether altering the controlled factor leads to a significant difference between the mean values obtained. If this is evaluated for each factor and interaction coefficient, an estimate of the main and interaction effects can be determined. The size of each coefficient in the model indicates its relative contribution to the overall response. Division of each coefficient by its estimated error gives the t-statistic for the null-hypothesis that the coefficient is statistically equal to zero. This value may then be compared with t-distribution tables in order to decide whether to accept or reject the hypothesis. In standard statistical computer packages an α-value (the confidence level at which the calculated t equals that in t-distribution tables) is also reported to allow significance to be easily recognized.

One of the assumptions of ANOVA is that the uncontrolled variation is random. However, measurements made over a period of time may produce a trend in the response. As a result the uncontrolled errors are no longer random since successive errors are correlated which can lead to a systematic error in the results. This problem is simply overcome by using a technique of randomization[321] where prior to running, the experimental order is completely randomized. This random order of experiments ensures that the errors in any factor are due to uncontrolled variables which are random. One disadvantage of complete randomization is that it fails to take advantage of any natural subdivisions in experimental material. For example, in a relatively large design there may be many combinations of factors requiring many different experiments. These may not be able to be completed in a single day but have to be

divided into several consecutive days. These subdivisions of the total design are called "blocks" and the process by which they are made "blocking".

3.1.2 Fractional Factorial Designs

One of the main disadvantages of full or complete factorial experimental designs is that the number of experimental runs required to estimate all the main effects and interactions increases rapidly as the number of factors increases. For example a full factorial design, at two-levels, requires 2^f runs (where f is the number of factors). Therefore, if six factors are to be examined, 64 experimental runs are required in which each run represents a different combination of factors. Additionally, this figure does not include any replicate experiments used to estimate the error in the model. When the number of factors is large the number of complex interactions involving three or more factors becomes extremely large. Although they can be estimated, they are often not significant and more often these higher-order effects are very small in comparison to main effects and two-factor interactions.[322] For most experimental situations main effects tend to be larger than two-factor interactions which in-turn tend to be larger than three-factor interactions and so on, so that at some point higher-order interactions can be regarded as negligible. Also, when there is a large number of factors in a full factorial design, it often occurs that only a few are significant. Fractional factorial designs use both of these aspects of factorial designs by disregarding the possible importance of high-order interactions, and use only a fraction of the experimental runs required for the complete design.

The main advantage of fractional factorial designs is that they can be used to investigate the effects of a large number of factors in a very few runs. This may be useful at the early stages of an investigation where little is known about the system and where fractional factorial designs are capable of deciding which factors are important. The simplest fractional factorial design is that of the half-fraction factorial design, represented as 2^{k-1} for a two-level design with k factors. The design is so called because it reduces the number of experiments by half the number required for the full design. When the information on higher interactions can be sacrificed, it should be made scientifically. Either it should be known that particular interactions are very small or deemed to be unlikely in the system under study. This is because fractional factorial designs lose their ability to uniquely estimate all of the model coefficients, or lose their "orthgonality". A half-fraction factorial design is usually made by first constructing a full factorial design for 2^{k-1} factors. Therefore for three

factors ($k = 3$) a full 2^2 design is constructed. Then the value of the k^{th} parameter is set to the product of the k^{-1} parameters. However, when the design is expanded and the interaction terms are constructed it is found that some of the factor combinations are identical to one another and are said to be "confounded" with each other. These terms cannot be used in the design and therefore it is usually only the main effects which are of use in fractional designs. If further fractionation is required (for example 2^{k-2} or higher) then more confounding will occur and lower interaction terms will be disregarded. However, there is an increased risk that if the main effect is large it may not be due entirely to the factor but to one which it is confounded with. Therefore, when designing fractional factorial experiments the main effects must be confounded with the highest possible interactions.

3.1.3. Star Designs

Although full factorial designs are useful for estimating first-order factor effects and interaction effects between factors they do suffer from three serious weaknesses.[323] The first is that often a very large number of experiments is required to plan a full factorial design. If a four factor, four level design in considered, a full design would require 256 experiments, which is clearly impractical and far more experiments than necessary for a sensible model (although fractional factorial designs are capable of dealing with this problem). A second difficulty is that there is no replicate information built into the design and so no estimate of experimental error. Replicates must therefore be added to the design by the experimenter and further increase the number of experiments required. Also factorial designs cannot be used to estimate additional second-order curvature effects such as those represented by the terms $\beta_{11}x_{1i}^2$ and $\beta_{22}x_{2i}^2$ in the model:

$$y_{1i} = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + \beta_{11} x_{1i}^2 + \beta_{22} x_{2i}^2 \qquad (3.5)$$

Therefore if the response is thought to contain any non-linear areas then a different approach to the experimental design must be used in order to investigate the possible curvature. A different class of experimental designs called "star" designs provide the information that can be used to fit models of the general form in equation 3.5.[317] Models of this class contain $2k+1$ parameters, where k is the number of factors included in the model. Star designs are located by a centre point from which other factor combinations are generated by moving a positive and negative distance in each factor dimension, one factor dimension at a time. Star designs therefore generate $2k+1$

factor combinations and are sufficient for estimating $2k+1$ parameters of models, which include curvature terms, such as equation 3.5. A star design with three-dimensional factor space is shown in figure 3.3.

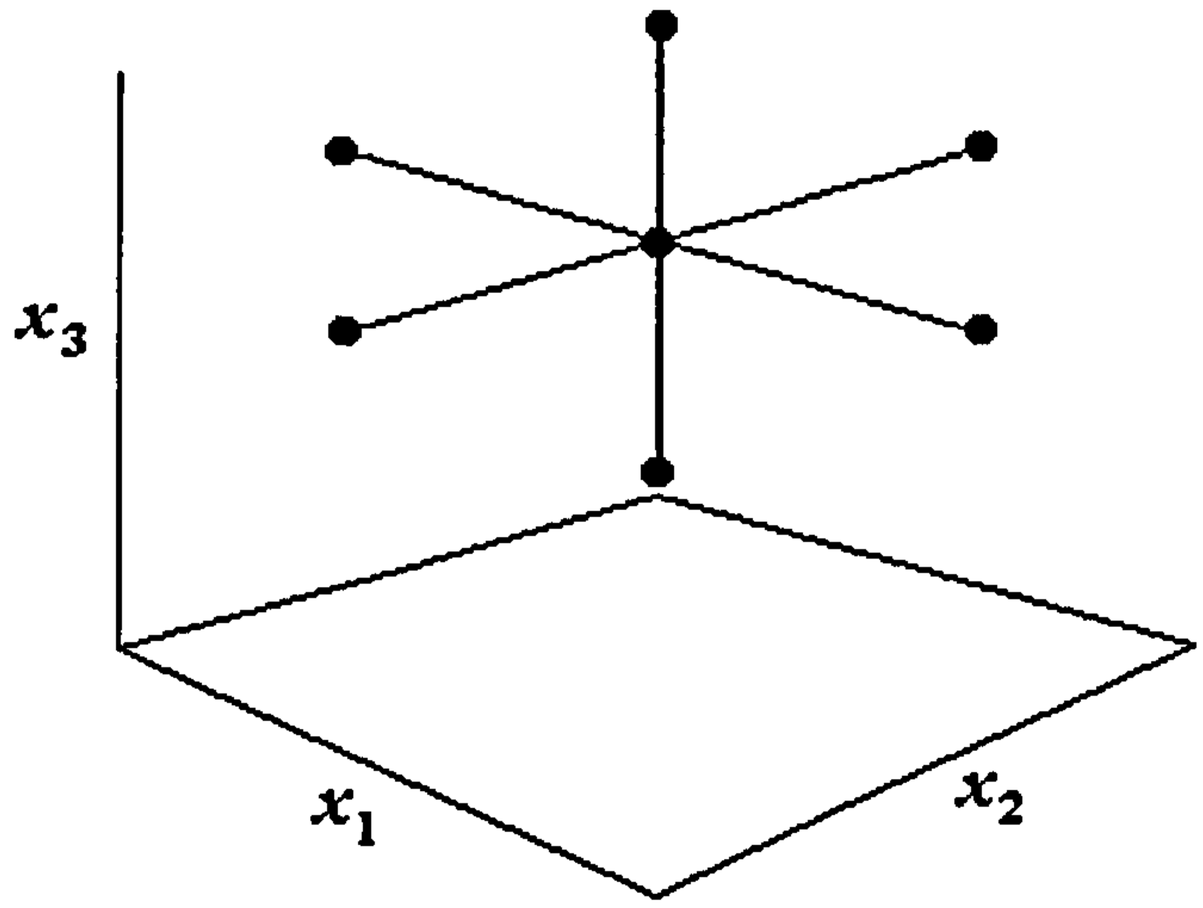


Figure 3.3 Factor Combinations for a Star Experimental Design in Three-Dimensional Factor Space.

Star designs are therefore capable of producing data that can be fitted to quadratic (squared terms) in the general equation (3.5), but unfortunately do not allow models possessing factor interaction terms to be used.

3.1.4 Central Composite Designs[317]

One of the most useful models for approximating a region of a multiple factor response surface is the full second-order polynomial model as it contains linear, quadratic terms (curvature) and interaction terms. For two factors, the model is shown in equation 3.6.

$$y_{1i} = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + \beta_{11} x_{1i}^2 + \beta_{22} x_{2i}^2 + \beta_{12} x_{1i} x_{2i} \qquad (3.6)$$

In general, if k is the number of factors being investigated, the full second-order polynomial model contains $\frac{1}{2}(k + 1)(k + 2)$ parameters. If a full second-order polynomial model is chosen, the appropriate experimental design for estimating the

$\frac{1}{2}(k + 1)(k + 2)$ parameters must be used. Two-level factorial designs are useful because they allow estimation of both the main and interaction effects. However, they do not allow the second-order curvature parameters to be estimated. Star designs are also an attractive possibility as they allow the estimation of the curvature effects as well as the main effects in the system. Unfortunately, they do not allow the estimation of interaction parameters and for all situations involving more than one factor, there are too few factor combinations to estimate all parameters of the full second-order polynomial model.

The combination of a two-level factorial design with a star design gives a composite design that is capable of estimating all parameters in full second-order polynomial models. If the centres of the two separate designs coincide, the resulting design is said to be a central composite design. A central composite design for three dimensional factor space is illustrated in figure 3.4.

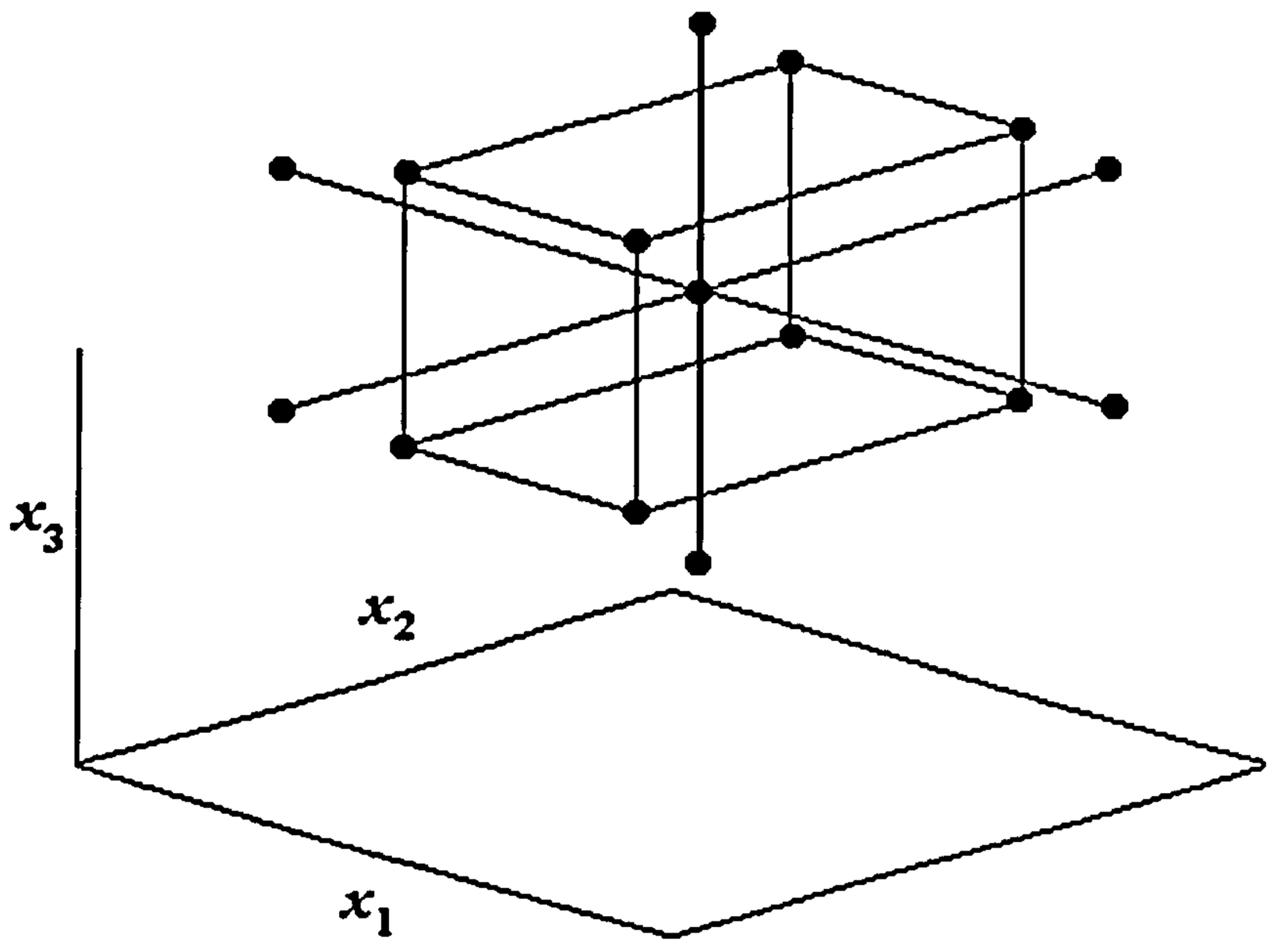


Figure 3.4 Factor Combinations for a Central Composite Experimental Design in Three-Dimensional Factor Space.

Replication is often included in central composite designs, with experiments being repeated four or five times. The results of the experiments are then used to give an estimate of the pure error in the system.

The level of error or uncertainty in a response surface obtained from a model is dependent upon the position of the experimental points in factor space. Thus in areas where experiments have been carried out there is less uncertainty in the surface when compared to regions where experiments have not been undertaken. The exact positions of the experimental points in a central composite design is therefore important with the distance the star points reach out from the centre to maintain orthogonality in the design, being calculated by equation 3.7.[324]

$$a^2 = \frac{\sqrt{(x_a + x_c + x_o)x_c} - x_c}{2} \qquad (3.7)$$

where, a is the distance of the star points from the design centre, x_a is the number of star points, x_o the number of centre points and x_c the number of cube points.

The term "orthogonal" in experimental design is used when the parameter estimates (β's) associated with any factor in a multifactor design are uncorrelated with those of another. Designs which do not have sufficient experimental points to fully describe the model can be made orthogonal by adding extra experimental points, often to the centre of the design.

The uncertainty in the generated response surface can be maintained at a constant level if the distance the cube and star points are from the design centre is equal. In this case the star points are moved closer to the centre and the subsequent designs are named "rotatable". When rotatable designs are used, the uncertainty predicted by a full second-order polynomial model is dependent only on the distance from the centre of the design and independent of the location of the experimental points. The equation that calculates the distance (a) for rotatable central composite designs is shown below.[322]

$$a^2 = \sqrt{x_c} \qquad (3.8)$$

where, x_c is the number of cube points.

The use of rotatable designs allow the errors associated with model to be constant within experimental factor space. Polynomial models used to fit response surfaces can be shown to fit experimental data extremely well but should not be extrapolated beyond the experimental domain.[325] A polynomial surface should be regarded only as an approximation to the data within the region covered by the experiment. Any prediction made from the polynomial about the response outside the region should be verified by experimentation before any assumptions are made.

3.2 Experimental Designs in Sample Preparation

Experimental design is useful for describing many chemical systems. It does, however, lend itself particularly well to analytical chemistry. The multiple variable systems often encountered within analytical chemistry would be extremely difficult to investigate and optimize using the conventional alter-one-factor-at-a-time approach. By using experimental design techniques many more variables can be examined much more efficiently while at the same time requiring far fewer experiments. The increased acceptance of statistically designed experimentation is illustrated by a recent review on the subject of chemometrics which contains over 1100 references, covering only the more significant developments in the field from December 1991 to December 1993.[326]

The field of SFE is particularly suited to experimental design methods as it is an extraction technique with many variables which may affect the extraction efficiency. Several examples in the literature use both factorial and central composite designs to investigate the parameters which affect analyte recovery, taken as the design response. A simplified 2^2 experimental design approach was used to optimize both extraction pressure and temperature for the determination of amine hydrochloride in avian feed.[327] The classical method of estimating main and interaction effects was used and found that extraction recovery was maximized at a low temperature and high pressure combination. A factorial design with three factors (2^3) has also been used to study pressure and temperature effects as well as the effect of the time of extraction on the recovery of 29 compounds, including PAHs and OCPs from a solid-phase extraction cartridge.[328] In this case ANOVA was used to show that pressure had the most significant effect on recovery followed by extraction time. The temperature was found to have little effect on the overall recovery.

A 2^3 factorial design was also used to optimize SFE parameters for the removal of oil from soybean seeds.[329] Supercritical carbon dioxide was used in the study which was modified with pentane. The three variables optimized in the study were pressure (limits between 50-150 atm), extraction temperature (limits between 60-100 °C), and extraction time (limits between 5-15 minutes), which required eight separate experiments. Extraction pressure was found to be the most significant factor followed by the time and the temperature of extraction. The effect of the interaction between factors was small and considered negligible. A 2^2 factorial experiment was then carried out which neglected the effect of temperature, since this was the least significant variable. Three repeat experiments were also undertaken at the design

centre. A linear equation was obtained from the design which was subsequently used to draw a response surface which was used to estimate the best experimental conditions. From the chemometric study, the best SFE operating parameters were found to be an extraction pressure of 150 atm, with a temperature of 100 °C and an extraction time of 15 minutes, which compared well with conventional Soxhlet extraction.

Experimental design was used to reduce the number of experiments required to study four main variables affecting SFE recovery of several steroidal compounds.[90] Pressure, temperature and flow-rate were studied as quadratics, with extraction time studied at only two levels as it was not possible to have a reduction on recovery on increasing the extraction time. The total number of experiments required (excluding replicates) was found to be 18, with the multivariate responses being evaluated using multiple linear regression. The overall results indicated that the density of the supercritical fluid had the greatest effect on steroidal solubility.

Central composite design techniques have been used to optimize temperature and pressure conditions for the SFE of hydrocarbons from spiked diatomaceous earth.[330] The procedure involved a two factor design giving nine different pressure/temperature combinations (excluding replication at the four cube points). Density, millilitres of CO_2 pumped and grams of CO_2 pumped were used as the design variables and evaluated over the various factor combinations. A full second-order polynomial model was fitted to the responses using linear regression with the adequacy of the fit and significance being determined by ANOVA. The results show that it is possible to obtain high recovery of simple hydrocarbons over a wide range of extraction conditions. The optimized parameters were then used to extract oil and grease from real soils with the results being comparable to standard methods using chloroflurocarbon as an extracting solvent.

A full second-order polynomial model was also used to optimize SFE extracting parameters including pressure, temperature, carbon dioxide flow-rate and particle size for the removal of essential oil from lavender flowers.[331] Each factor was investigated at five different levels requiring 30 experiments in total, which included six replicates at the design centre. The effects of the operating conditions of SFE on the relative overall yield was calculated with respect to steam distillation. Optimum SFE conditions were found to yield greater than 80 % recovery at relatively low pressure (85.77 bar) and temperature (36.58 °C), combined with a CO_2 flow-rate of 10.11 L h^{-1}

and a particle size of 2143 μm. The model was found to fit the data points with a minimum correlation coefficient of 0.8855 (for percent camphor in essential oil).

Experimental design protocol has not solely been restricted to analytical scale SFE. The effects of four process variables on the destruction efficiency of a flow through supercritical water oxidation reactor were investigated using factorial design experiments.[332] These process variables included; reactor throughput, concentration of surrogate waste (% acetone), maximum reactor tube-wall temperature, and applied stoichiometric oxygen. The analysis was conducted using a two-level factorial design, steepest assent method, and a central composite design. The experimentation identified a significant positive effect for stoichiometric oxygen applied and temperature variations between 400 and 500 °C. The increase in destructive efficiency due to stoichiometric oxygen provided strong evidence that supercritical water oxidations are catalyzed by excess oxygen. In addition, destructive efficiency was increased by increasing the Reynolds number and residence time within the reactor.

Aims of the Project

Often in organic analysis the most crucial part of the overall procedure is the initial sample preparation. Despite great advances in separation science, extraction of trace levels of organic compounds from complex matrices has shown little variation in many years.

Therefore the main initial aims of the project are:

- To investigate various analytical-scale sample preparation techniques capable of quantitatively removing low levels of organic chemicals from complex matrices. As the collaborating body responsible for funding is Analytical and Environmental Services Ltd (part of Northumbrian Water Group), the compounds under investigation are those which are thought hazardous to human health and therefore of environmental concern. In addition, the matrices studied are those routinely analyzed in an environmental laboratory and include both liquids and solids.

- In an attempt to reduce organic solvent consumption and in particular the use of chlorinated solvents, the sample preparative techniques examined are to be critically studied to assess their overall solvent usage. Where possible, methods are to be devised which totally eliminate or significantly reduce the consumption of organic solvents compared to the amount currently used for traditional extraction methods.

- As well as an overall drive to reduce solvent consumption, any measures to achieve faster analysis and therefore increase sample throughput are to be investigated. This is particularly relevant to organic sample preparation since this is often the rate determining stage in the overall analytical procedure.

- Unfortunately, many of the more traditional organic sample preparative techniques are incapable of being automated and therefore require large amounts of analyst time being spent handling large volumes of hazardous solvent. This is particularly true for aqueous samples. Consequently, the project shall assess the performance of sample preparation equipment which is, or shows some ability to be fully (or partially) automated.

EXPERIMENTAL

Chapter 4

Section A: Sample Preparation

4.1 Supercritical Fluid Extraction

The basic components of a supercritical fluid extraction system have been discussed in the introduction (section 2.3.1.2) and are in common with any SFE instrumentation. Two different SFE instruments were used throughout the project. The details of both the apparatus are given below.

a. The Carlo Erba SFE

(Sections 5.2, 5.4, 6.2, and 6.3)

The optimization of SFE extraction and collection conditions (section 5.2) were performed using a Carlo Erba SFE 30 extraction system (Fisons Instruments, Crawley, UK), depicted in figure 4.1. The system uses a 150 ml syringe pump (SFC 300) to deliver carbon dioxide at either constant pressure or flow-rate to the oven compartment (extraction unit) which contains the sample. An outer jacket is fitted to the syringe pump which is kept cool by using a recirculating refrigerant bath containing an ethanol/water mixture. This allows the carbon dioxide to be pumped as a liquid.

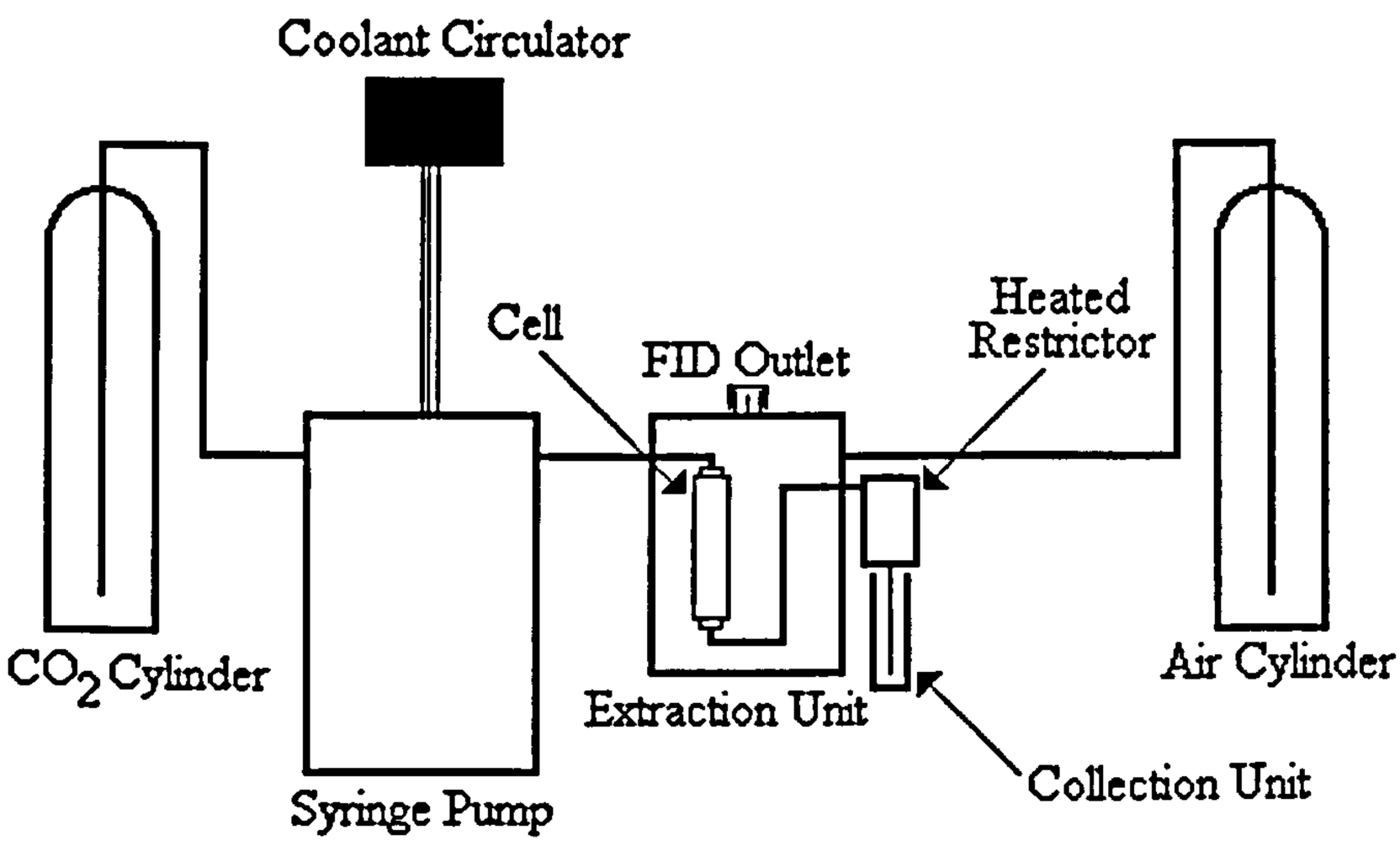


Figure 4.1 Schematic Diagram of the Carlo Erba SFE Apparatus.

All components of the apparatus are connected using standard 1/16 in. stainless-steel tubing. All SFE was performed in off-line mode (although the system was fitted with an on-line flame-ionization detector) with the back-pressure in the system being maintained by a heated stainless-steel tube restrictor (1/16 in.) which was crimped at its end to restrict the flow of supercritical carbon dioxide to approximately 2 ml min^{-1}. The restrictor was kept at a constant 150 °C throughout the extraction to prevent plugging by the formation of solid carbon dioxide during depressurization. Two 10 port Valco valves are housed in the oven compartment which allow the extraction cell to be flooded with carbon dioxide whilst stopping flow to the restrictor (static extraction). To perform a dynamic extraction, the second of the two valves is opened by means of an air actuated switch which allows flow through the restrictor. All extractions were done in constant pressure mode with the syringe pump maintaining pressure by executing small changes in the flow-rate. The optimization experiments were carried out using a 1.67 ml Keystone stainless-steel extraction cell (Mettler-Toledo, Halstead, Essex, UK) which was hand tightened. The Carlo Erba SFE unit described is also used for the solid-phase extraction-SFE approach to extraction from water discussed in section 5.4 and for the selective extraction procedures shown in section 6.2 (also used in part of the selective extraction procedure in section 6.3). Only slight modification was required from the apparatus described above in that the size of the solid-phase extraction disks necessitated the use of a larger extraction cell. A Jasco (Mettler-Toledo) 10 ml stainless-steel extraction cell was used for these extractions.

b. The Jasco SFE

(Sections 5.3, 6.3, 7.5, and 8.2)

Due to the large size of the headspace extraction cell used for the direct extraction of organochlorine pesticides from water (section 5.3), the Carlo Erba SFE 30 could not be used because of restricted oven space. A Jasco SFE system (shown in figure 4.2) was therefore utilized for the direct SFE extraction of pesticides from water.

The Jasco SFE apparatus consists of a SFE/SFC back-pressure regulator, BPR (model 880-1) which is coupled to modular Jasco reciprocating pumps (880-PU) and an oven (860-CU).[333] The system uses two pumps with the second pump, used for the addition of modifier, being controlled by the main carbon dioxide pump. The main pump is cooled by a refrigerant head attachment in which an ethanol/water mixture is recirculated from a cooling bath. The carbon dioxide cylinder is connected to the main

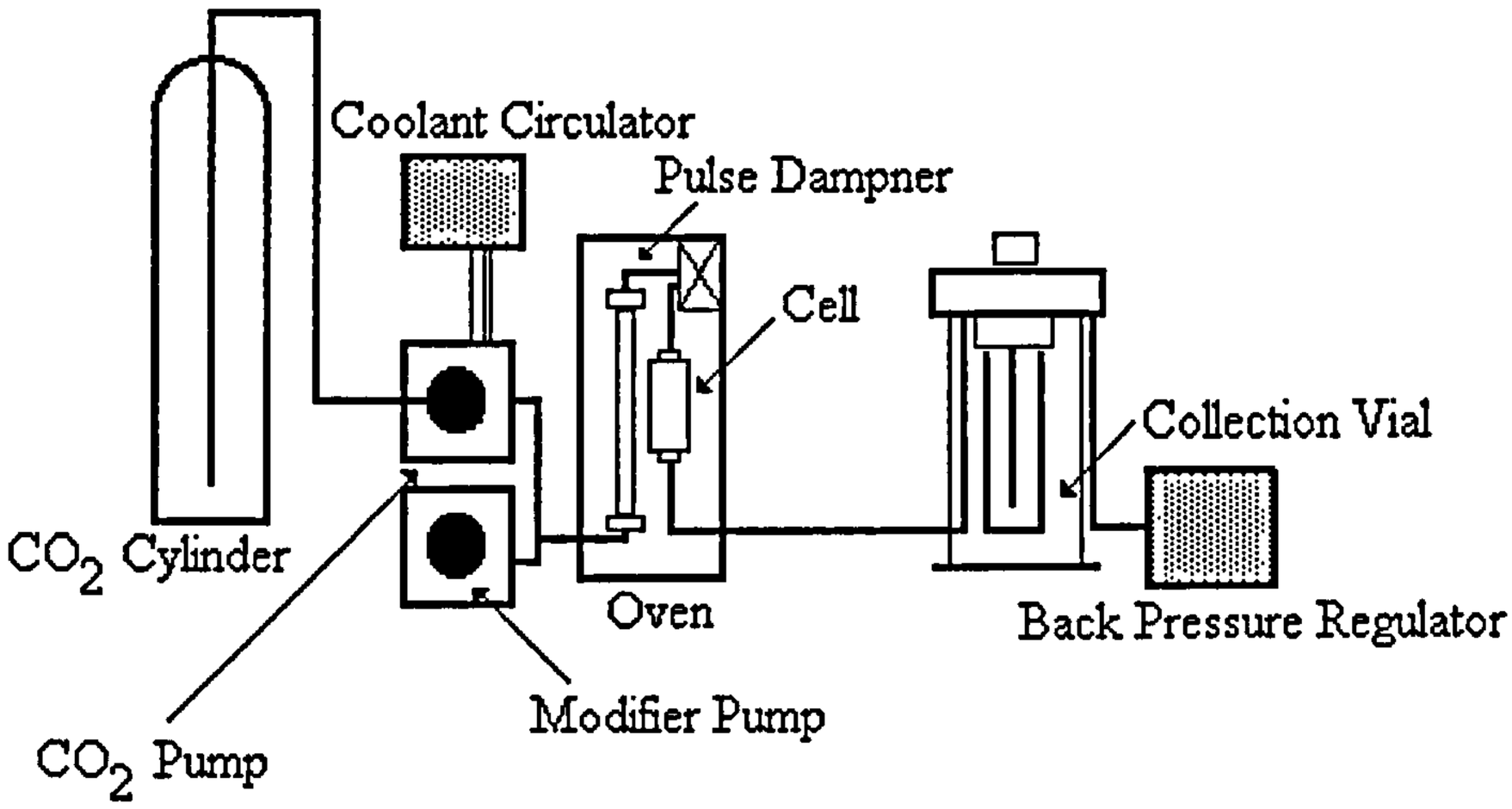


Figure 4.2 The Jasco SFE.

pump by approximately 2 m of 1/16 in. stainless-steel tubing which is immersed into the cooling bath to reduce the temperature of the fluid prior to pumping. The second pump allows an exact concentration of modifier to be added to the carbon dioxide continually during the extraction. The arrangement is similar to a gradient HPLC system with the two flows being mixed in a high pressure relief valve (Rheodyne 7037). As with the Carlo Erba apparatus, the units in the Jasco SFE are connected *via* 1/16 in. stainless-steel tubing. A Rheodyne 7010 switching valve is used to divert the path of the supercritical carbon dioxide to the extraction cell which is housed in the Jasco oven compartment. The oven also contains a pulse dampner and 2 m of stainless-steel tubing in which the fluid flows prior to entering the extraction cell. The pulse dampner reduces the uneven flow produced by the reciprocating pumps and facilitates (together with the excess tubing) the heating of the supercritical carbon dioxide prior to introduction to the sample.

The system pressure is maintained by the BPR based on a pressure transducer and a regulating valve which is electronically controlled. The regulating valve itself is an ordinary solenoid valve consisting of a needle which is driven by a solenoid into a valve seat. A cross-sectional view of the valve is shown in figure 4.3. The valve controls the supercritical fluid flow by changing the gap between the valve needle and seat at high speed. By periodically opening and closing the flow-path, the arrangement is capable of maintaining the required pressure, which is monitored by the pressure transducer. This type of regulator does not suffer from plugging as precipitated solids and dry ice from carbon dioxide are always being tapped and forced to pass through the valve. In addition, unlike the Carlo Erba SFE apparatus, the system allows the

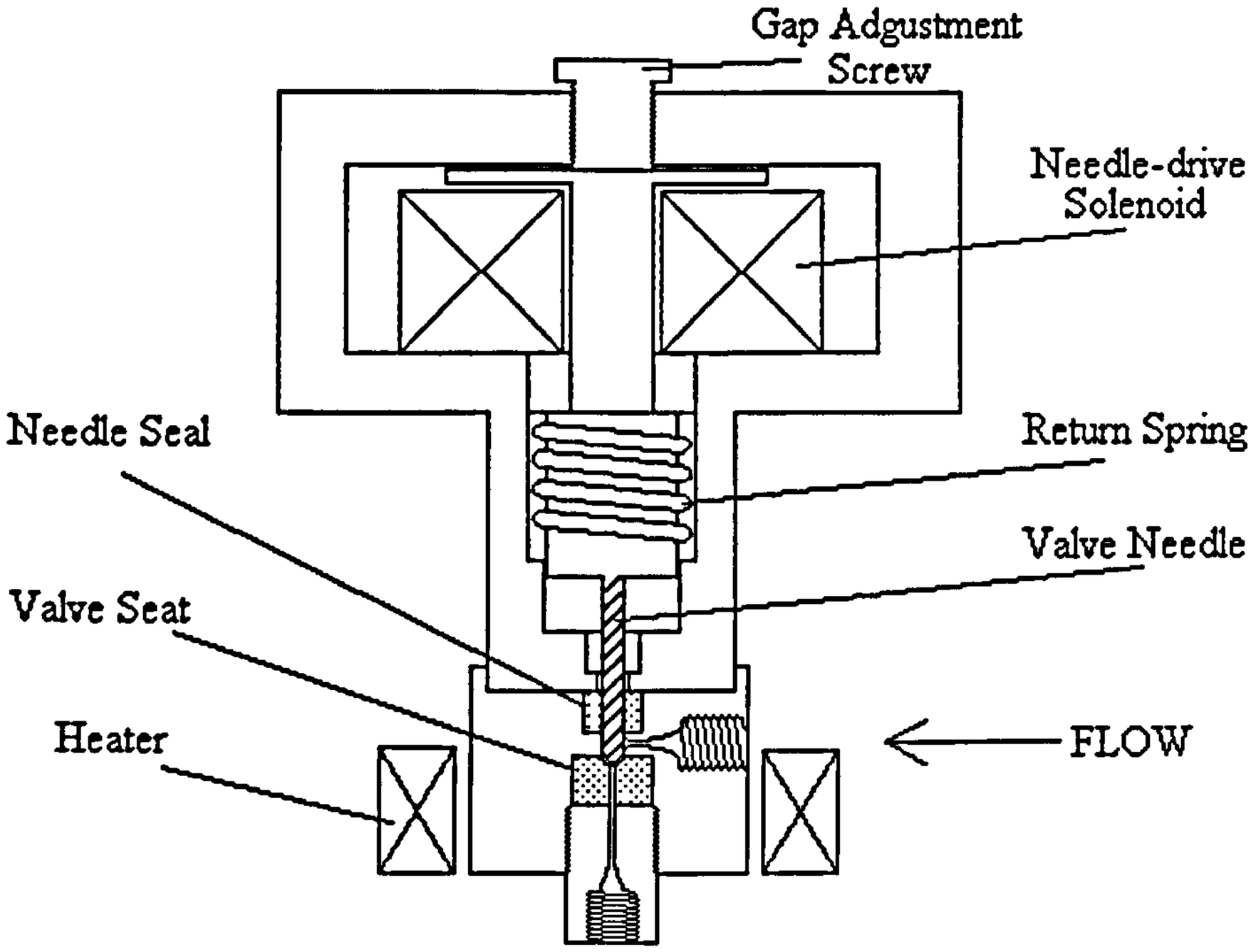


Figure 4.3 The Jasco Back-Pressure Regulator.

independent control of both flow-rate (set at the pump) and the extraction pressure (set at the BPR).[333]

The Jasco SFE has been used successfully to selectively extract organochlorine pesticides from herbicides in section 6.3 and for the supercritical fluid extraction of polycyclic aromatic hydrocarbons in section 7.5, where 2.5 ml extraction cells (Phase Separations) were used throughout. In addition, it was the preferred instrument for the SFE of selected pesticides from characterized soils (section 8.2).

c. SFE Collection Vessels

The descriptions of the SFE apparatus above are incomplete in so much as no mention of their collection units has yet been made. Modification of both the Carlo Erba and Jasco collection units was deemed necessary because of the inability of the commercially supplied models to allow efficient recovery of extracted analytes. The problems associated with both collection units are discussed in greater detail in section 5.2. The basic Carlo Erba collection device is depicted in figure 4.4.

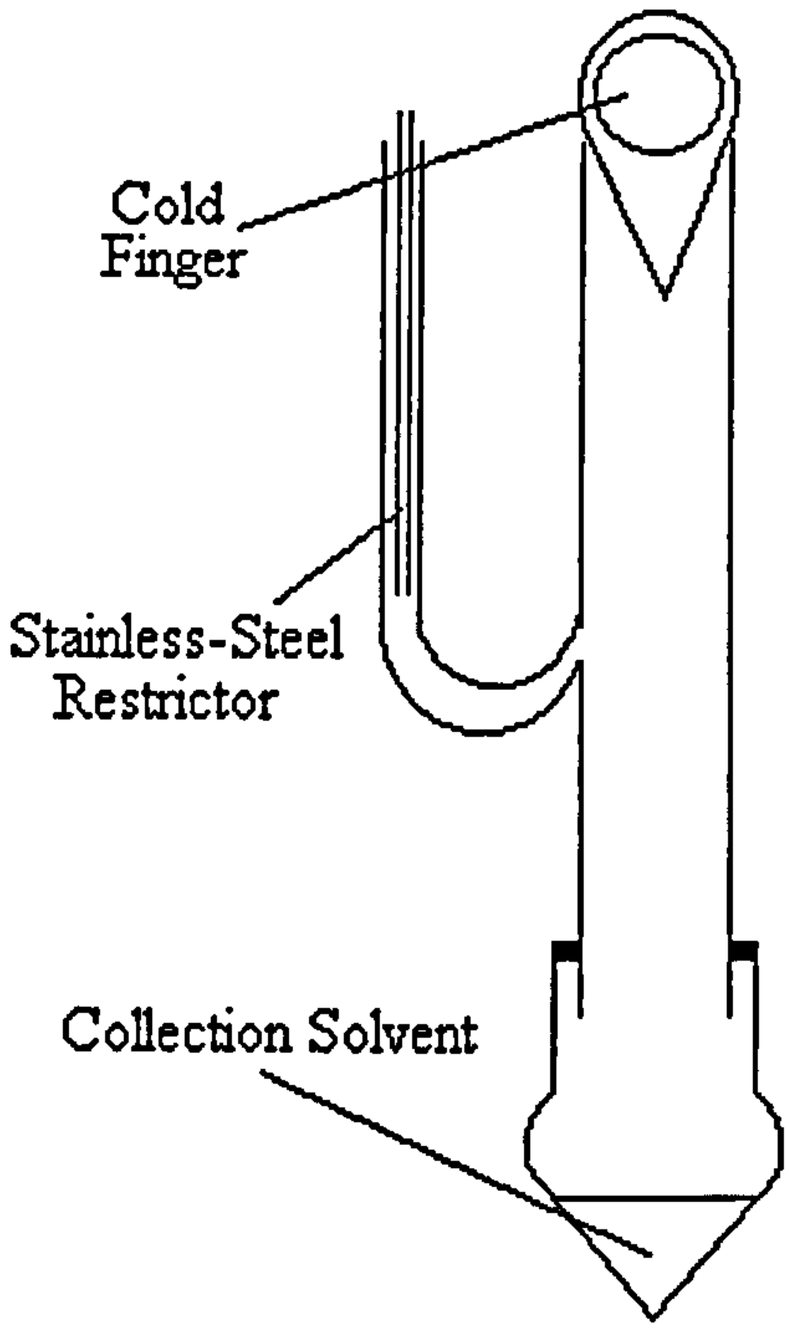


Figure 4.4 Diagram of the Carlo Erba Collection Unit.

The crimped stainless-steel restrictor, from the SFE 30 unit, is inserted into a glass side-arm tube (3 mm i.d.) which is connected to a larger tube (150 cm in length with a 12 mm i.d.), fitted with a pear-shaped flask (8 cm^3) and cold finger. A small amount of solvent is placed in the bottom of the flask prior to extraction. The cold finger is present in an attempt to prevent losses of extracted analytes by aerosol formation when the supercritical carbon dioxide is depressurized.

The commercially supplied Jasco collection unit simply consists of a tapered test-tube (105 cm in length with a volume of 10 cm^3) which is held in a metal frame and pushed against the base of the BPR by a spring at the bottom of the frame. The design is shown in figure 4.5.

In this design, no attempt is made to reduce the possibility for analyte loss at the top of the test-tube. Several different modifications were evaluated including extending the 1/16 in. stainless-steel to the bottom of the test-tube and the use of septa at the top. Unfortunately, no small changes in the design of either collection unit resulted in more efficient performance and it was decided to use a new design in an attempt to completely resolve the loss of extracted analyte by aerosol formation. The new design is shown in figure 4.6 and consists of a 25 cm^3 glass screw-cap vial which is sealed with a plastic top containing a rubber septum, coated with PTFE on the underside (both supplied by Phase Separations, Clwyd, Wales). The septum is pierced with the

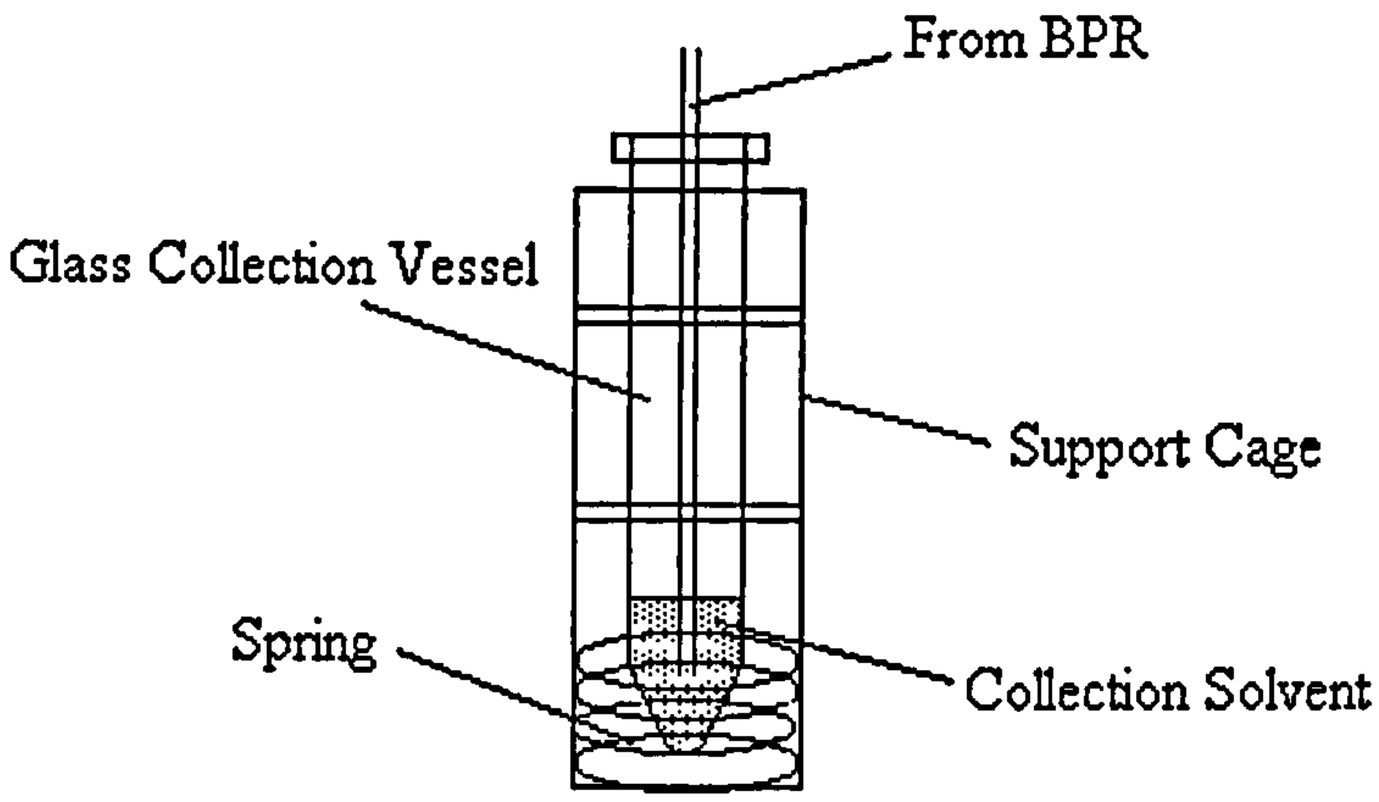


Figure 4.5 The Jasco SFE Collection Unit.

stainless-steel tube from the BPR (or restrictor) and also with a hypodermic needle which allows the depressurized carbon dioxide to escape. A few millilitres of a suitable collection solvent are added to the vial prior to the commencement of extraction. The hypodermic needle is also fitted with a solid-phase extraction cartridge (Waters or Varian) containing 100 mg of C_{18} packing material, through which the carbon dioxide passes.

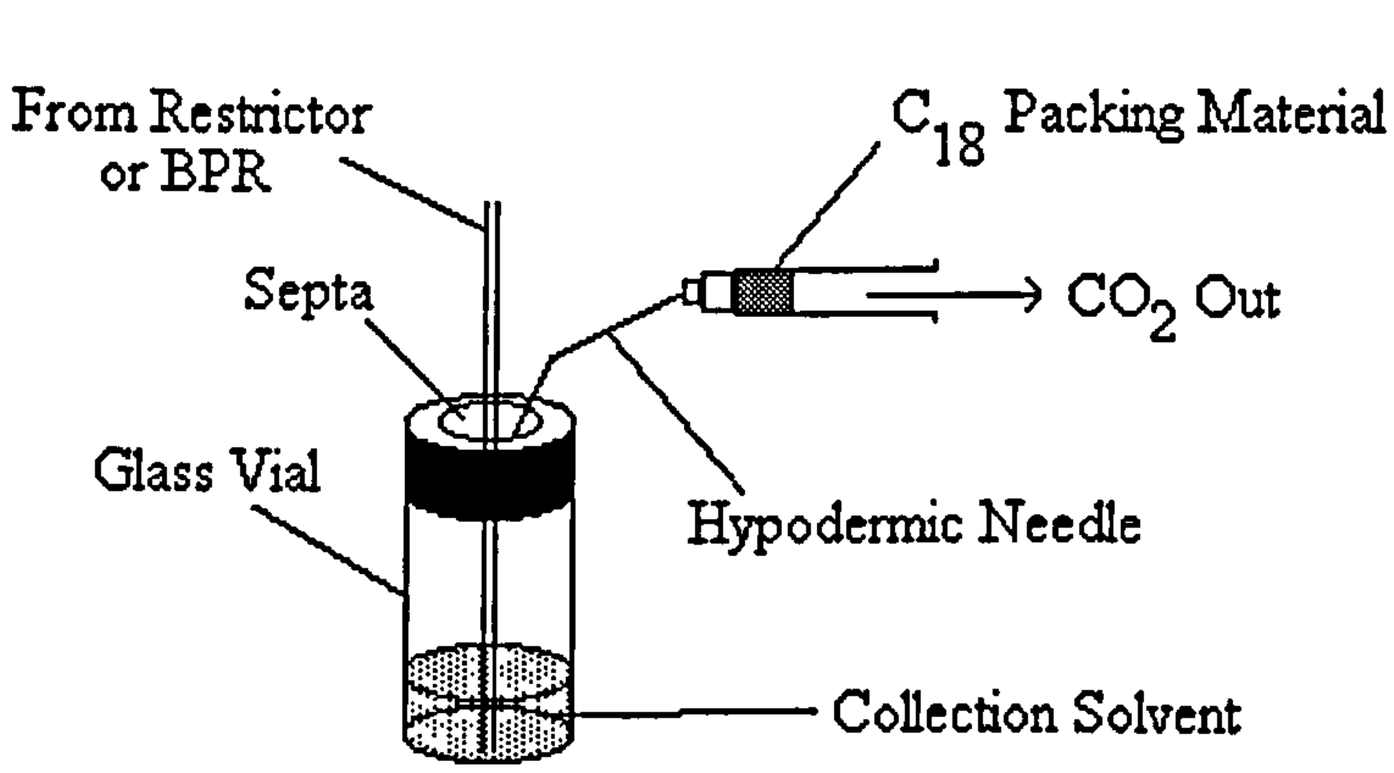


Figure 4.6 Schematic Representation of the Modified SFE Collection Unit.

A C_{18} sorbent was chosen to allow relatively non-selective adsorption of a range of both non-polar and moderately-polar analytes. Once the cartridge had been pre-conditioned with 2-3 ml of methanol it was capable of trapping analytes that were removed from the collection solvent by the aerosol action of the depressurized carbon dioxide. The benefits of the new collection unit are discussed in greater detail in section 5.2.

d. The Jasco "Headspace" Extraction Cell

The direct extraction of pesticides from a water matrix (detailed in section 5.3) was accomplished using the modified Jasco SFE extraction cell shown schematically in figure 4.7. The cell is made from 12 mm thick stainless-steel with an internal volume of 50 cm^3. The cell top screws onto the base with a 24 mm section of threading coated with PTFE to ensure a pressure-tight seal. The seal is also maintained by a PTFE O-ring placed between the cell top and base. Carbon dioxide enters the cell from the top and is diffused through the aqueous sample by means of a conventional HPLC solvent filter, normally used for helium sparging, attached to the carbon dioxide inlet tube which is extended to the bottom of the cell. The porous 10 μm filter is used to increase the mixing of the supercritical fluid with the sample and has been found to aid analyte recovery.[194] The "headspace" configuration of the cell is different to that of the "flow-through" cell and allows the supercritical carbon dioxide to pass through the sample before exiting at the top of the cell. The outlet is covered by a frit which allows only dissolved analytes to leave the cell, preventing water "splash-over".

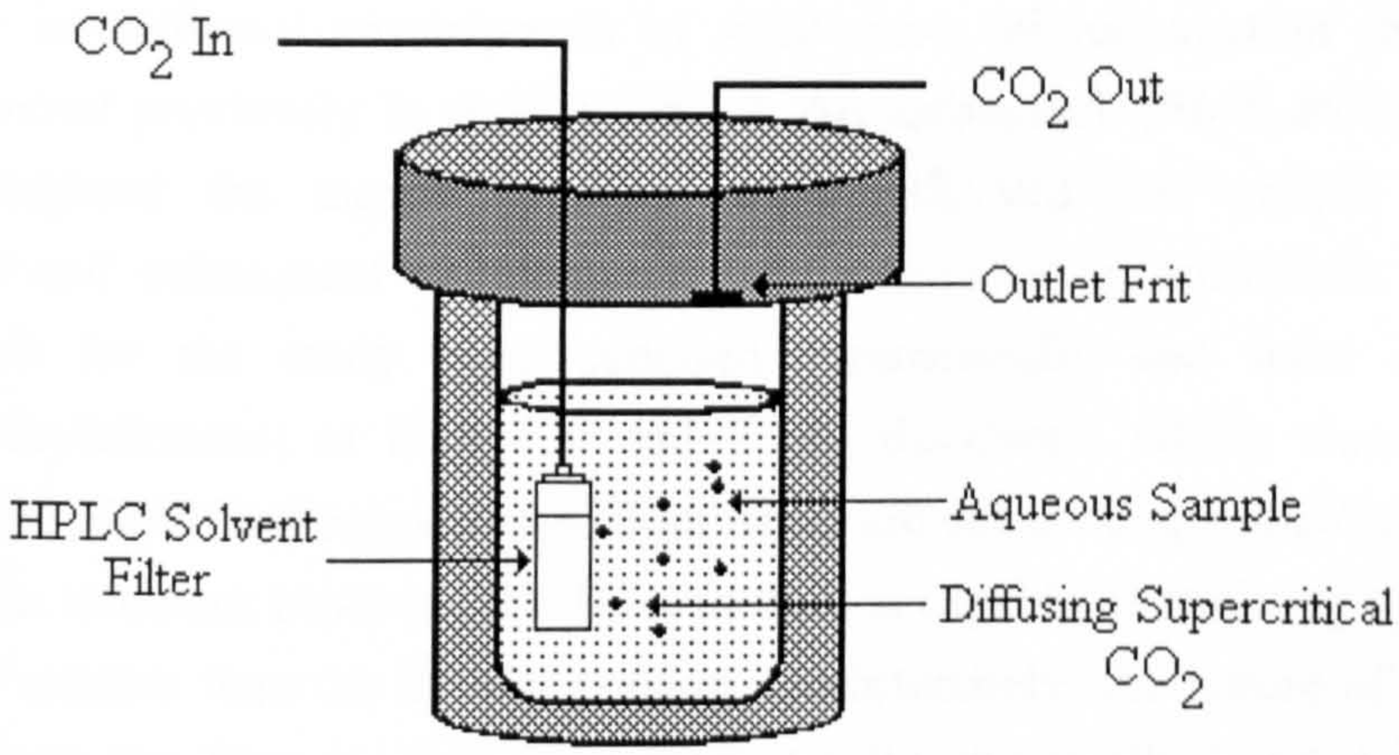


Figure 4.7 Schematic Diagram of the "Headspace" Extraction Cell.

4.2 Solid-Phase Extraction

Solid-phase extraction has been utilized in two different forms within the research, both in conjunction with SFE.

Solid-Phase Extraction Columns

The use of solid-phase extraction columns in preventing the escape of analytes from the SFE collection vessel has already been discussed in the previous section. In

addition, the advantages of their use are detailed in greater length in section 5.2. Extraction cartridges containing 100 mg of C_{18} sorbent packing were used throughout the SFE experimentation and were obtained from two different sources. Varian Bond-Elut SPE cartridges were supplied by Phase Separations whilst Millipore Sep-Pak cartridges were supplied by Fisons.

Solid-Phase Extraction Disks

Membrane extraction disks (Empore) consisting of octadecyl particles enmeshed in a network of PTFE have been used for the indirect supercritical fluid extraction of pesticides from water and are described in the SPE instrumental section (2.3.2.2). The disks used for the study were 47 mm in diameter and were used together with a standard Millipore glass filtration unit and vacuum. The extraction disks used were obtained from either Jones Chromatography (Glamorgan, UK) or Phase Separations.

4.3 Solid-Phase Microextraction

The basic instrumental requirements of solid-phase microextraction (SPME) have been discussed previously in section 2.3.3.2. An automated SPME arrangement was used throughout the experimentation, which allowed the sample adsorption, desorption and subsequent GC analysis to be controlled by computer. The coated fibres used for the study were supplied commercially and were coated with poly(dimethylsiloxane) at both 100 and 7 μm thickness', which were colour co-ordinated for identification purposes. Both fibres are designed specifically for analysis of non-polar analytes, however, the 100 μm fibre is capable of retaining a much larger amount of analyte than the thinner version. Unfortunately, the nature of the 100 μm fibre prevents the fibre coating from being totally chemically bonded to the silica support and it has a tendency to be partially removed at high desorption temperatures. Therefore, the 100 μm fibre has a maximum operating temperature of 220 °C, above which the coating becomes unstable. The fibre is designed for the removal of volatile organic compounds (VOCs) from aqueous samples which easily desorb at temperatures far below the maximum operating temperature.[270,274] In comparison, the thinner 7 μm fibre coating is more strongly chemically bonded to the silica support and is capable of being desorbed at far greater temperatures, having a maximum operating temperature of 320 °C. This allows the fibre to be used for the extraction and analysis of semi-volatile organic compounds (SVOCs) from aqueous samples, although the thinner coating is obviously not capable of removing the same

concentrations of analytes which can be extracted using the 100 μm fibre. The design of the fibre and fibre holder is depicted in figure 4.8.

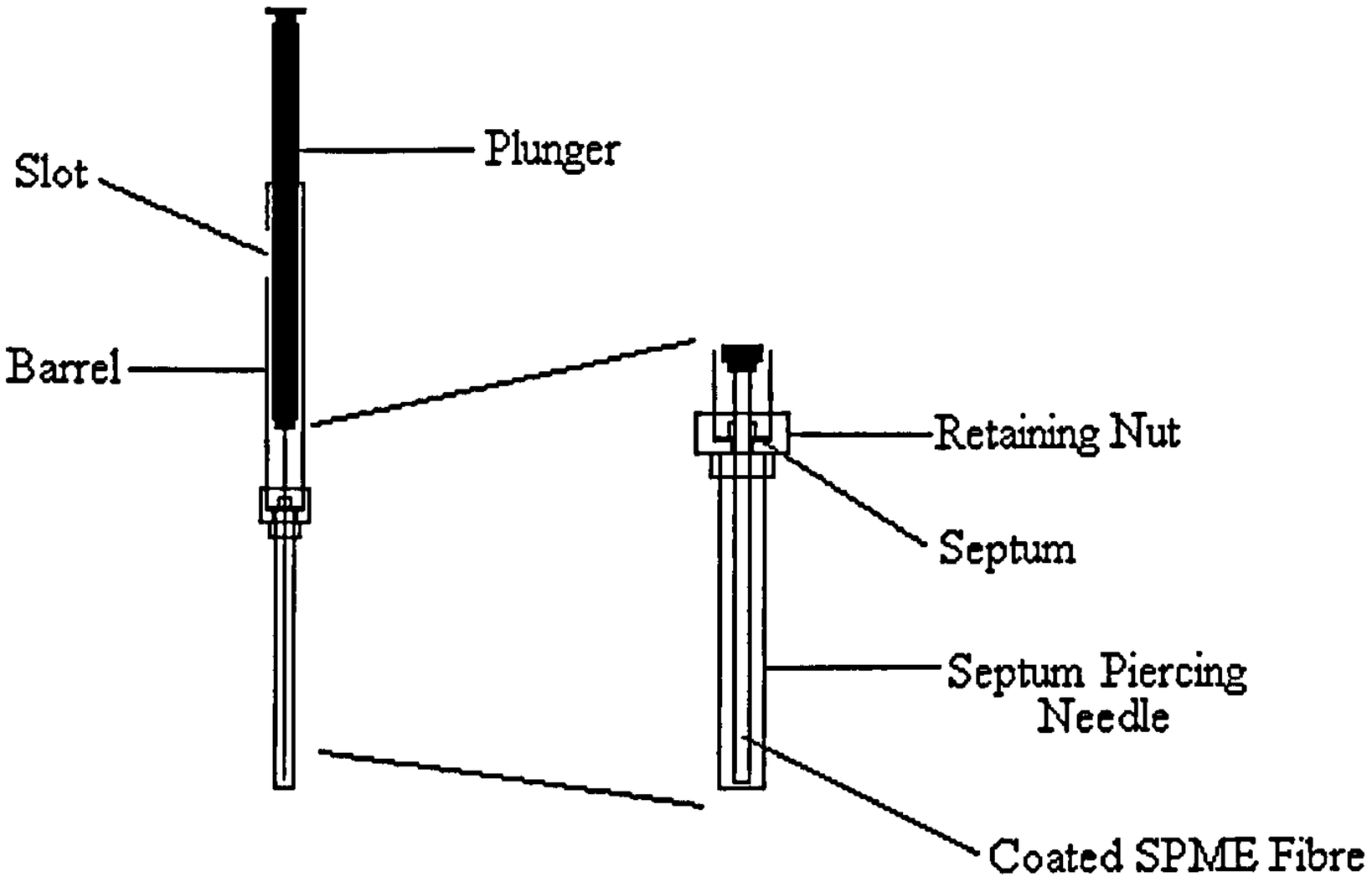


Figure 4.8 The Commercial SPME Fibre Assembly.

The fibres were designed only for use in automated systems and were used together with a commercial fibre holder (both fibre and holder supplied by Supelco, Poole, Dorset, UK). To accommodate the fibre, the septum piercing needle shown in the diagram has a slightly wider internal diameter than a standard GC injection syringe and is of similar length (5 cm). The replaceable fibre is attached to the holder assembly by screwing into the base of the plunger after which the retaining nut is replaced. The slot in the side of the fibre holder is used to allow the colour of the fibre to be observed. A Varian 8100 autosampler (Walton-on-Thames, Surrey, UK) was used to accommodate the SPME fibre for automation. The standard syringe assembly was removed from the autosampler and replaced with the SPME holder containing the fibre. The autosampler was used together with a Varian 3400 gas chromatograph with a conventional split/splitless injector. The aqueous samples to be extracted were contained in 2 ml glass vials, sealed with a PFTE septa and cap (supplied by Chromacol, Herts., UK) and were filled to a pre-set level depending on whether liquid or headspace sampling was to be performed. A more detailed description of the GC operating conditions is given in the chromatography section of this chapter.

The SPME autosampler was controlled by software through a PC which allowed adsorption and desorption times to be set, whether headspace or liquid sampling was required and the number of vials to be automatically analyzed (described in section

4.11). Only minor modification of the entire autosampler/GC was required including exchange of the syringe assembly for the SPME holder, installation of the Labview software (Varian) to control the autosampler and connection of the PC to the autosampler.

The main advantage of the commercial SPME assembly over a "home-made" version is its ability to perform fully automated analysis of multiple samples. This was achieved using a standard GC autosampler which was controlled using specialist software from a PC. Once the adsorption and desorption times, sampling mode (liquid or headspace) and the option for multiple or single vial analysis had been input into the software, the autosampler routine was as follows. The carousel containing the samples was moved forward, rotated under an optical sensor to count the number of vials and retreated. The fibre on the SPME assembly was then fully protruded and quickly returned to ensure no faults were present. The carousel was once again moved under the SPME assembly and the sheathed fibre allowed to pierce the sample vial septa. Once inside the vial, the fibre was extended a pre-set amount depending on whether liquid or headspace sampling was selected, where it remained for the set adsorption time. Upon completion of the adsorption period, the fibre was again sheathed inside the protective needle and the whole syringe carriage moved to retract the fibre from the sample vial. The carousel was then retreated to expose the injection port and the needle placed in the hot split/splitless injector where once again the fibre was protruded from its protective sheath, where it remained for the set desorption time. At the end of the desorption time, the GC temperature programme was automatically started together with the Star Workstation integrator (Varian). When multiple samples were extracted, a pre-adsorb delay could be input into the SPME software which allowed the fibre to begin adsorption in the next sample before the GC temperature programme had finished from the current sample. This allowed considerable time-savings when long adsorptions were being used.

Before any extractions could be performed using a new SPME fibre it first had to be conditioned in the split/splitless injector at a temperature above that which is to be used for routine desorption, but below the maximum operating temperature of the fibre. The fibre pre-conditioning is used to remove any coating which could "bleed" during desorption and is similar to the routine GC capillary column pre-conditioning required before their use. For experiments involving the 100 μm fibre, a blank desorption temperature of 220 °C was chosen as this was the highest temperature which could be used without the possible removal of the poly(dimethylsiloxane) coating. The fibre was conditioned in the injector for a minimum of three hours at this

temperature, with the split vent open, to fully remove any contaminants which may have caused high baseline noise or ghost peaks. After this initial conditioning stage, the fibre was repeatedly injected into the GC until the resulting chromatogram was clear from any contamination. A fifteen minute blank desorption was also carried out each morning prior to extraction to ensure any airborne interferences adsorbed when the fibre was left unused overnight in the laboratory atmosphere, were removed.

4.4 Soxhlet Extraction

Details of a Soxhlet extraction system can be found in section 2.2.2.1. Soxhlet extractions were performed using a "QuickFit" Soxhlet apparatus of 40 cm^3 internal volume (supplied by S.H. Scientific, Blyth, Northumberland, UK) which in turn was fitted with a 250 ml round-bottomed flask and a reflux condenser. An isomantle was then used to heat the flask, which contained 100 ml of solvent (dichloromethane). The sample was contained in a cellulose extraction thimble of dimensions 80 x 22 mm i.d. (Whatman, Maidstone, UK), which was plugged with a wad of cotton wool to prevent escape of the sample. Both the extraction thimble and cotton wool were pre-extracted with dichloromethane before use to remove possible contamination.

4.5 Microwave-Assisted Extraction[290]

The recent developments of microwave sample preparative techniques for organic analysis have been discussed in section 2.3.4. A MES-1000 commercial microwave instrument (CEM Corporation, Buckingham, UK), designed specifically for use with organic solvents, was used throughout the microwave extraction work. The system consisted of a PFTE coated microwave cavity in which a turntable, capable of holding twelve samples vessels, was placed. During an extraction, the turntable rotated back and forth through 180 ° to prevent the various tubes attached to the sample vessels being caught in each other. Extraction conditions were controlled by temperature or pressure using an in-built fibre-optic thermodetector (which allowed extraction temperatures to be selected from 20 - 200 °C) and a pressure transducer (reading pressures from 0 - 200 p.s.i.), respectively.

Several safety features were built into the system specifically to deal with the problems associated with the use of flammable solvents and to prevent their ignition. The main safety feature was the solvent vapour detector which was incorporated in the

systems air exhaust and which turned off the microwave magnetron if solvent vapours were detected in the microwave cavity. The exhaust blower was used to continually move air through the cavity and failure or blockage of the air flow also resulted in the magnetron power supply being cut.

All sample vessels used were double-walled in design and consisted of an inner liner constructed from Teflon-PFA together with an outer body made from special grade Ultem® poly(etherimide) that is resistant to attack by organic solvents and gives the mechanical strength to the vessel design. The sample, together with the extraction solvent were placed in each inner liner which had a volume of 100 cm^3. The cap of the vessel was also lined with Teflon-PFA and contained a Teflon rupture membrane pressure rated to 200 p.s.i. If the safety membrane broke during heating, solvent vapours could escape through a small port and were carried *via* an 1/8 in. PTFE tube to an expansion container. The container was situated in the centre of the turntable and was air sealed to prevent loss of the hot solvent vapours into the microwave cavity. A 1/4 in. PTFE tube connected the expansion container to the external exhaust where any solvent vapours were carried by the venturi effect. The external exhaust was then placed in a laboratory fume hood to safely remove any solvent vapours. The basic layout of the safety features in the MES-1000 are shown in figure 4.9.

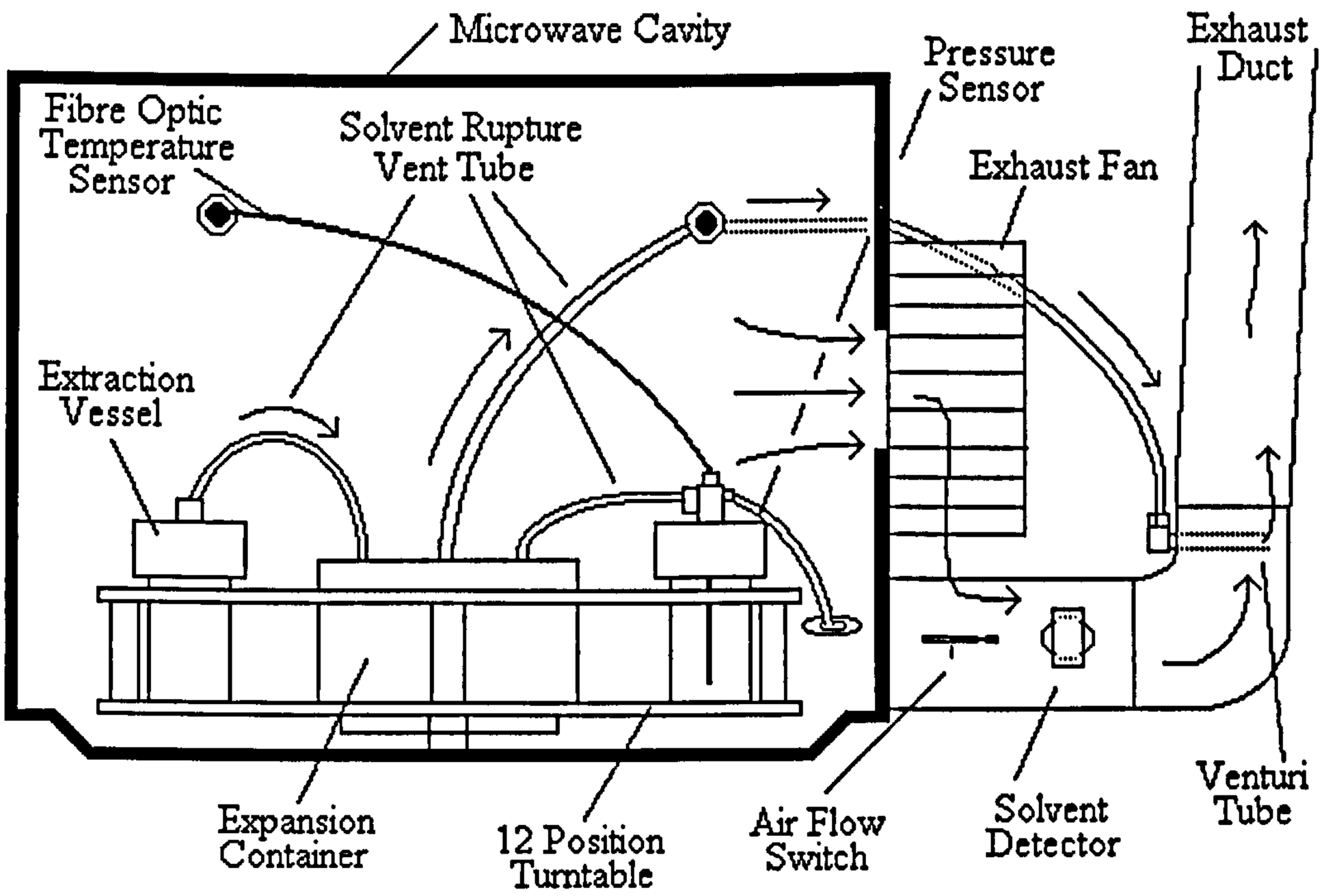


Figure 4.9 The Basic Safety Features of the Microwave Solvent Extraction System.

One of the twelve vessels used contained a modified cap that allowed a fibre-optic temperature detector and an in-line pressure sensor to be used *in-situ*. The optical fibre was housed in a Pyrex tube which protected it from solvent attack. A 1/4 in. PTFE tube was also connected directly to the sensor vessel which was used to measure the pressure inside the vessel. This tube was filled, prior to each extraction, with distilled water and the pressure the column of water exerted on a pressure transducer used to measure pressure inside the vessel. This system allowed the temperature and pressure to be monitored during an extraction. The extraction was then controlled by either the set temperature or pressure depending on which parameter reached its programmed set-point first. The microwave sample vessel design is illustrated in figure 4.10.

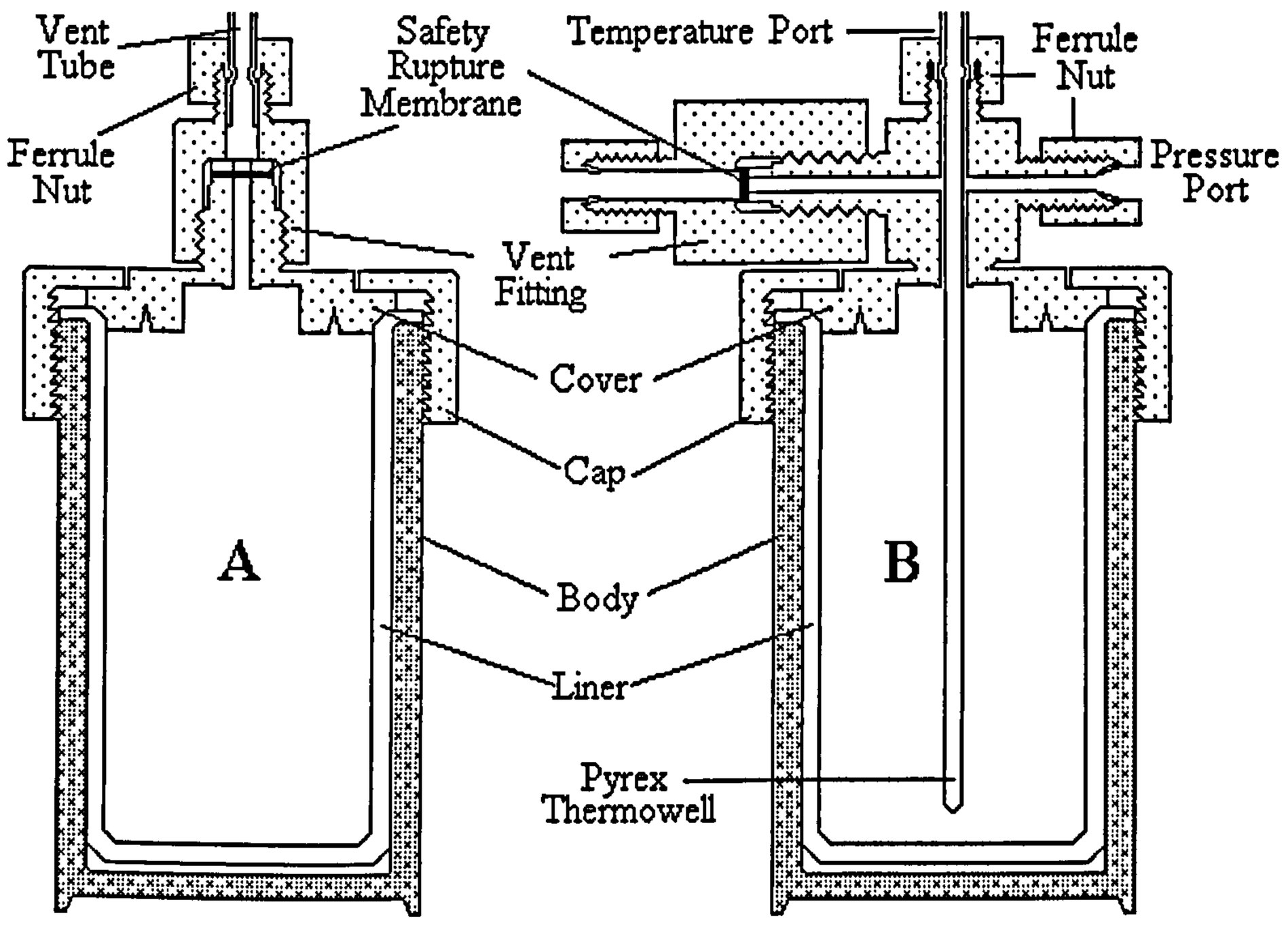


Figure 4.10 Microwave Extraction Vessels.
(A= Standard Extraction Vessel; B= Sensor Vessel showing temperature and pressure ports).

Section B: Chromatographic Analysis

4.6 Gas Chromatography with Electron Capture Detection

Gas Chromatography with Electron Capture Detection (GC-ECD) was used to analyze organochlorine pesticide extracts obtained during experimentation detailed in sections

5.2 - 5.4. ECD detection is favoured for organic compounds containing halogen functions since the detector uses a β-electron emitting source (^{63}Ni) whose electrons are sensitive towards electronegative halogen atoms. The ECD is one of the most sensitive GC detectors available and is routinely used for trace OCP and PCB analysis. The great sensitivity of the detector towards halogenated organics means that chlorinated solvents are not compatible and must not be injected into a GC-ECD system. A more detailed description of the detector is given in section 2.4.1.

Analysis of the extracts obtained in the SFE work detailed in chapter 5 was performed by a Perkin Elmer 8420 gas chromatograph with electron capture detection (Buckinghamshire, UK). 0.5 µl of the hexane extract or calibration standard was manually injected onto a 12 m x 0.25 mm i.d. x 0.25 µm BP-5 fused silica column (SGE, Ringwood, Australia) through an injection port maintained at 250 °C. A split flow of 50 ml min^{-1} was operated during the injection with a 1 minute delay where upon the split vent was closed. Separation was obtained isothermally with an oven and detector temperature of 240 °C and 350 °C, respectively. Nitrogen was used as both carrier and make-up gas and operated with a column head pressure of 20 p.s.i. Under the above conditions, analysis was performed in less than 9 minutes. A set of five calibration standards between 0 and 1.0 µg ml^{-1} was used to assess system linearity and to calibrate the instrument. The range of standards was run each day together with the top standard which was used as a continuing calibration check every 5 samples. Hexabromobenzene (at the 1 µg ml^{-1} level) was used as an internal standard and was added to every sample and standard.

A typical GC-ECD chromatogram for a 1 µg ml^{-1} standard (containing lindane, aldrin and dieldrin) is shown in figure 5.1 (chapter 5).

4.7 Gas Chromatography with Nitrogen / Phosphorus Detection

As with GC-ECD, gas chromatography with nitrogen / phosphorus detection (GC-NPD) is a specific analysis tool for the separation and determination of organic compounds containing nitrogen or phosphorus atoms. The degree of selectivity between the two classes of compound is dependent on the set-up of the GC system. However, in this case, where GC-NPD is used to analyze the thermally desorbed triazine herbicides obtained during SPME, it is the nitrogen specificity which is of use and the NPD is used in the NP mode. A further description of the NPD is found in section 2.4.1.

As described in section 4.3, a Varian 3400 GC fitted with a conventional split/splitless injector, NPD (termed thermionic specific detector by Varian) and equipped with an Varian 8100 autosampler was used throughout the SPME experimentation without modification. The GC was fitted with a 30 m x 0.25 mm i.d. x 0.25 μm film thickness DB-5 capillary column (J & W Scientific, supplied by Phase Separations). Pre-drilled Thermogreen injection septa (Supelco) were used throughout the analysis to reduce septa coring and bleed during the desorption stage (the SPME syringe assembly has a slightly wider-bore needle than conventional GC injection syringes). Various injection and initial column temperatures were investigated during the study (detailed in section 5.5), however, the detector settings, gas flow-rates, pressures and temperature programme remained constant. A detector temperature of 310 °C together with a NPD bead current of 3.100 A was kept constant throughout experimentation. Also the inlet pressure of 15 p.s.i. helium and the make-up gas velocity of 30 ml min^{-1} nitrogen, with 5.5 ml min^{-1} hydrogen and 175 ml min^{-1} air, were all fixed. The temperature programme used to elute the analytes focused at the front of the GC column after desorption was as follows; variable initial column temperature, hold time 1 minute, then 15 °C min^{-1} to 150 °C (hold 0.5 minutes), finally 4 °C min^{-1} to 210 °C. During the desorption stage, the split vent remained closed to allow quantitative transfer of all analytes to the front of the chromatographic column. However, the split vent was opened 1 minute after the temperature programme was initialized to sweep the injection port and allow removal of any residual compounds.

Chromatograms illustrating the use of the NPD are shown in section 5.5.

4.8 Gas Chromatography with Mass Selective Detection

Gas chromatography utilizing mass selective detection (GC-MSD) is now accepted as the most convenient way in which to separate and analyze complex mixtures of volatile and semi-volatile organic compounds. Unlike the detectors already mentioned, the MSD is non-specific and shows a response for a great variety of compounds. In addition to its non-selective nature, identification of the compounds detected by the MSD is also possible by reference to the mass spectra it produces. Two different MS configurations are routinely coupled to GC in a "bench-top" arrangement. The quadrupole mass spectrometer is the most common and is the type used throughout the research, although an ion-trap mass spectrometer has been shown to be of use in many applications (see SFE and SPME application sections). Both types of instrument are capable of being run in both total ion and selected ion modes

of operation. Total ion chromatograms (TIC) are of use for the identification of unknown peaks as the whole mass range is scanned every cycle and have been used in all the applications described in the initial method development. However, selected ion monitoring (SIM) is an inherently more sensitive technique, since only pre-selected ions (abundant in the component of interest) are analyzed and has been used where sensitivity was a problem. A more detailed discussion on the modern-day role of GC-MSD is found in section 2.4.1.

GC-MSD analysis has been used extensively throughout the research under three separate operating conditions.

(i) GC-MSD Analysis in Selective Extraction from an Aqueous Matrix

Identical analysis conditions were used throughout the experimentation described in chapter 6, with the exception of the ions selected for quantitation. A listing of the appropriate ions for each compound is given in table 4.1. The first column of ions are those chosen to quantitate the extract and are usually the most abundant ion in the samples mass spectrum, with the remaining two columns of ions used as qualifiers.

Analysis of the extracted analytes (throughout the work detailed in chapter 6) was performed with GC-MSD using a Hewlett Packard GC, Model 5890, with MSD, Model 5971A (Hewlett Packard, Bracknell, UK). 0.5 µl of the extract was manually injected into a split/splitless injection port (250 °C) and swept onto a HP-1 fused silica capillary column of dimensions: 25 m x 0.20 mm i.d. x 0.32 µm film thickness (Hewlett Packard). The GC temperature programme was as follows: initial oven temperature, 85 °C; held in split mode 0.75 minutes (split flow 40 ml min^{-1}). A linear ramp was then used at 16 °C min^{-1} to a final temperature of 285 °C which was held for a further 2 minutes. Helium was used as the carrier gas with a column head pressure of 9 p.s.i. After separation, the analytes were passed to the MSD through a heated transfer line maintained at 280 °C which was also used to heat the MSD ionization source. An electron energy of 70 eV together with a multiplier voltage of 2150 V was used throughout the analysis. Data acquisition was started after a solvent delay of 3 minutes.

In both cases (section 6.2 and 6.3), quantitation was achieved using the internal standard method to account for injection errors. For the selective SFE of organochlorine and organophosphorus pesticides (section 6.2), demeton-s-methyl (at a

Compound	Quantitation Ion	Qualifier Ion (1)	Qualifier Ion (2)
OCPs			
Lindane	183	181	219
Aldrin	263	293	66
Dieldrin	263	277	79
Heptachlor	272	100	337
Isodrin	193	263	147
OPPs			
Dichlorvos	109	185	79
Diazinon	137	179	304
Malathion	173	125	93
Chlorfenvinphos	267	269	323
Internal Stds.			
Demeton-S-methyl	88	109	142
β-Endosulphan	195	237	159

Table 4.1 Quantitation and Qualifier Ions used in GC-MSD Analysis of Pesticides.

concentration of 20 μg ml^{-1}) was used as the internal standard and was added to each extract and standard. A calibration range between 0 and 20 μg ml^{-1} was used to quantitate the extracts and was run each day together with the 20 μg ml^{-1} standard which was used as a continuing calibration check and ran every 5 samples. A different internal standard (β-endosulphan at 10 μg ml^{-1}) was used in the selective extraction of OCPs from herbicides (section 6.3) and was added to all samples as well as to the calibration standards which ranged from 0 to 10 μg ml^{-1}. The standards and extracts were analyzed in the same way as those described previously.

Chromatograms illustrating the use of GC-MS for the determination of OCPs and OPPs are shown in section 6.2. A typical chromatogram obtained during the analysis of the OCPs in section 6.3 is shown in figure 6.5.

(ii) GC-MSD Analysis in the Extraction of Polycyclic Aromatic Hydrocarbons from Contaminated Land

The analysis of PAH extracts throughout the work detailed in chapter 7 was performed on one of two GC-MSD instruments.

Initial experimentation using a contaminated land sample (soil 1) containing relatively low overall concentrations of PAHs (extracted using Soxhlet and microwave assisted extraction) was performed on a Varian 3400 GC interfaced with a Finnigan Incos 500 quadrupole mass spectrometer (Hemel Hempstead, UK) and equipped with a Finnigan A200S autosampler. A 30 m x 0.25 mm i.d. x 0.25 μm film thickness DB-5 capillary column (J&W Scientific) with a helium head pressure of 15 p.s.i. was used to achieve separation of the extract using the following temperature programme: initial column temperature, 85 °C; hold for 3 minutes; then 6 °C min^{-1} to 300 °C; hold 7 minutes. The split / splitless injector was maintained at 300 °C and operated in splitless mode for 1 minute prior to opening the split valve. The split flow was 40 ml min^{-1}. The mass spectrometer transfer nozzle was heated to 270 °C with the ionization source maintained at 150 °C. The electron energy and multiplier voltage were set at 70 eV and 1500 V, respectively.

The analysis of both the high concentration composite soil and the CONTEST soil (section 7.3 - 7.5) was carried out on a Hewlett Packard 5890 Series II GC fitted with a Hewlett Packard 5972A MSD and 7673 SFC/GC autosampler. The injector and transfer line temperatures, temperature programme and capillary column where identical to those described above. The initial head pressure was set at 15 p.s.i. and a constant flow of carrier gas (1 ml min^{-1}) was maintained automatically throughout the temperature programme using electronic pressure control (EPC). An electron energy of 70 eV together with a multiplier voltage of 1850 V was used throughout the analysis.

The following procedure was used for both GC-MS instruments :-
Prior to injection, 500 μl of extract (250 μl for samples extracted using Soxhlet) was added to an autosampler vial together with 250 μl of internal standard. The vial was then capped and placed in the autosampler which injected 2 μl of the mixture into the GC injector. The mass spectrometer was operated in selected ion monitoring mode (ions used for quantitation shown in table 4.2) with the data acquisition being started after 4 minutes. A perfluorotributylamine (PFTBA) calibration gas was used to automatically tune the instruments as necessary. A five point calibration plot containing 2, 5, 10, 20 and 50 μg ml^{-1} individual PAHs and 20 μg ml^{-1} internal standard mix was prepared and used to establish the system linearity. The 10 μg ml^{-1} standard was run after every ten samples, as a continuing calibration check and used to calculate response factors for each compound. Concentration of individual PAHs was assessed against the response of the two internal standards chosen to elute near the beginning and end of the chromatogram.

PAH	Quantitation Ion	Internal Standard
Acenaphthene	153	3,6-Dimethylphenanthrene
Acenaphthalene	152	3,6-Dimethylphenanthrene
Anthracene	178	3,6-Dimethylphenanthrene
Benz(a)anthracene	228	6-Ethyl Chrysene
Benzo(b)fluoranthene	252	6-Ethyl Chrysene
Benzo(k)fluoranthene	252	6-Ethyl Chrysene
Benzo(ghi)perylene	276	6-Ethyl Chrysene
Benzo(a)pyrene	252	6-Ethyl Chrysene
Chrysene	228	6-Ethyl Chrysene
Dibenz(a,h)anthracene	278	6-Ethyl Chrysene
Fluoranthene	202	3,6-Dimethylphenanthrene
Fluorene	166	3,6-Dimethylphenanthrene
Indeno(1,2,3-cd)pyrene	276	6-Ethyl Chrysene
Naphthalene	128	3,6-Dimethylphenanthrene
Phenanthrene	178	3,6-Dimethylphenanthrene
Pyrene	202	3,6-Dimethylphenanthrene
3,6-Dimethylphenanthrene	206	-
6-Ethyl Chrysene	256	-

Table 4.2 Quantitation Ions used for Analysis of PAH Extracts together with their Appropriate Internal Standards.

A typical GC-MS selected ion monitored chromatogram (Hewlett Packard) for the 16 PAHs (at the 10 μg ml^{-1} level) and two internal standards (20 μg ml^{-1}) is shown in figure 7.1 (chapter 7).

(iii) GC-MSD Analysis in the SFE of Selected Pesticides from Characterized Soils

The organochlorine and organophosphorus pesticides listed in table 4.1 (with the exception of chlorfenvinphos as a replacement for dichlorvos) which were extracted from characterized soils by SFE were analyzed using the Hewlett Packard GC-MSD described in (*ii*) above. The instrument was operated in SIM mode under the following conditions: 2 μl of the extract was injected onto a 30 m x 0.25 mm i.d. x 0.25 μm film thickness DB-5 capillary column (J & W Scientific) through a split/splitless injection port kept at 280 °C. The initial injection was performed in splitless mode with the split vent (40 ml min^{-1}) opened after 1 minute. Helium was

used as a carrier gas at a head pressure of 9.7 p.s.i. and operated in constant flow mode (1 ml min^{-1}). The detector transfer line was maintained at 290 °C throughout the analysis. A temperature programme identical to the one described in (*i*) was used to separate the pesticides. Quantitation was achieved using an external calibration set of standards ranging in concentration from 0 to 1 μg ml^{-1}. β-endosulphan (1 μg ml^{-1}) was again used as an internal standard and added to each sample and standard.

Typical GC-MS chromatograms for both OCPs and OPPs are shown in figures 8.1 and 8.2, respectively (both at the 1 μg ml^{-1} concentration level).

4.9 High Performance Liquid Chromatography

High Performance Liquid Chromatography (HPLC) is commonly used for the analysis of organic compounds which cannot be analyzed by GC. These analytes include those which are thermally labile or those which are sufficiently non-volatile that they cannot be vaporized in a GC injection port. However, for HPLC the only restriction is that the sample must be dissolved in a solvent. HPLC is examined further in section 2.4.2.

HPLC was used for the analysis of herbicide extracts in sections 6.3 and 8.2. Although s-triazine herbicides are capable of being separated and detected by GC and are routinely analyzed using GC-MS, phenylurea herbicides are thermally labile and break down at temperatures commonly used in GC split/splitless injection ports. They therefore cannot be analyzed by GC without some form of derivatization, for example by methylation of the amide NH group to form a stable tertiary amide.[334]

Identical analysis conditions were used in both sections to analyse both s-triazine and urea herbicides simultaneously and utilized reversed-phase HPLC. A Gilson (Anachem, Luton, Beds., UK) reciprocating pump, Model 305, was used to isocratically pump a 55 % methanol-water mixture (1 ml min^{-1}) through a C_{18} column with dimensions 25 cm x 4.6 mm i.d. (Phase Separations). The column was maintained at 35 °C in a column oven (Gilson). Samples were introduced into the system *via* a Rheodyne 7125 injection valve fitted with a 10 μl loop. Detection of the herbicides was by a UV-vis spectrophotometer (Jasco, Model UV 975) set at 240 nm. A LDC/Milton Roy integrator (Thermo Separations, San Jose, CA, Model CI 10) was used for peak analysis. The concentration of the extracts was determined by external calibration using a range of standards from 0 to 1 μg ml^{-1} which were injected daily

together with the 1 μg ml^{-1} standard which was used as a check standard every 10 samples. An internal standard was found not to affect the precision of the analysis and therefore not used.

Chromatograms illustrating the use of HPLC for the separation and quantitation of s-triazine and urea herbicides are shown in section 6.3.

Note:- The chromatograms shown in section 6.3 are identical to those obtained during the soil study in chapter 8.

4.10 Reagents

Gases

SFC grade carbon dioxide was used throughout the SFE experimentation as the supercritical fluid. The CO_2 was purchased from Air Products (Sunderland, UK) as a liquefied gas in stainless steel cylinders with a purity of ≥ 99.995 %.

All gases used in gas chromatography (nitrogen, helium, air and hydrogen) were purchased from BOC gases (Birtley, Tyne and Wear, UK).

Standards

i Organochlorine Pesticides

Lindane, aldrin, dieldrin, heptachlor, and isodrin were purchased as solids from Promochem Chemicals (St. Albans, UK) with a minimum purity of 99.5 % from which 1000 μg ml^{-1} stock solutions were prepared in acetone.

ii Organophosphorus Pesticides

Dichlorvos, diazinon, malathion, and chlorfenvinphos were purchased from Promochem Chemicals as solids at a minimum purity of 99.0 % and also made into 1000 μg ml^{-1} stock solutions with acetone.

iii Herbicides

s-Triazine herbicides (atrazine, simazine, propazine, trietazine) and urea herbicides (chlortoluron, isoproturon, diuron) were all purchased, as solids, from Promochem Chemicals at a minimum purity of 99.0 %. The standards were subsequently made into 100 μg ml^{-1} stock solutions with methanol.

iv Polycyclic Aromatic Hydrocarbons

Sixteen different PAHs were used, purchased as a prepared stock solution (Sigma Chemicals, Poole, UK) at a concentration of 2000 μg ml^{-1} in a benzene: dichloromethane (1:1) solvent.

v Internal Standards

Hexabromobenzene (used as an internal standard in sections 5.2 - 5.4) was again purchased from Promochem Chemicals at a purity of 99.2 %. Demeton-s-methyl and β-endosulphan (used as internal standards for all GC-MS analysis of pesticides) were also purchased from Promochem Chemicals at purities of 99.0 % and 99.5 %, respectively. 3,6-Dimethylphenanthrene and 6-ethylchrysene (used as internal standards in all PAH analysis) were purchased as solids from Lancaster Chemicals (Lancashire, UK) and used to prepare a 2500 μg ml^{-1} stock solution in methanol.

Solvents

Acetone, dichloromethane, hexane, and methanol were obtained from two sources at HPLC grade, BDH (Merck, Poole, UK) and Rathburn (Walkerburn, UK). HPLC grade water (used in all SPME experimentation) was purchased from J.T. Baker (Berks., UK).

Miscellaneous

Celite (diatomaceous earth) used in the SFE optimization experiments described in sections 5.2 and 8.2 was purchased at GPR purity (60-80 mesh size) from BDH Chemicals. Sodium chloride, used in the direct extraction from water using SFE, was also purchased at GPR grade from the same supplier.

GF/A glass membrane filters (used to remove particulate matter still present after microwave extraction) were purchased from Whatman (Maidstone, UK). Cellulose Soxhlet thimbles were also obtained from the same supplier.

The commercial blender, used in the contaminated land soil sample preparation described in chapter 7, was purchased from Waring (S.H. Scientific, Blyth, Northumberland, UK).

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identification of all unknown peaks. The software was linked to a spreadsheet (Microsoft™ Excel) which allowed the complete automation of the data handling, from initial peak integration to calculation of unknown concentrations by interpolation of calibration graphs and assessment of internal standard performance. Overall quantitation was achieved by the use of previously determined quantitation ions which were selected to be abundant in the sample mass spectrum.

4.11 Software

Statistical Software

Chemometric studies involved in the optimization of microwave and SFE operating variables were aided using a specially designed computer program. The DOS™ based "Design Expert" (Version 3.0, Stat-Ease Inc., Minneapolis, USA) allowed the variables to be input at their maximum and minimum constraint values and then allowed calculation of the experimental points needed to describe a specified design. A central composite design was chosen to optimize three and four operating variables for extraction using microwave energy and SFE, respectively, which required 20 (three variables) and 30 (four variables) experiments, including repeat extractions at the centre of the design. The software allowed the entire set of experiments to be broken down into blocks, that were completely randomized and which could be performed on different days. In addition, the software automatically coded the experimental points allowing easy interpretation of results. Once the experiments had been performed, the response obtained for each experimental data point could be input into the design and a quadratic model calculated. The model could then be statistically evaluated to determine whether it was capable of adequately describing the chemical system. As well as a mathematical description, Design Expert also allowed the construction of response surfaces for one and two variables which gives a graphical interpretation of the effect the variables have on the system response. Design Expert is used for the chemometric studies described in sections 7.4 and 7.5.

Chromatographic Software

Automated SPME was controlled by Labview software (Varian) incorporated in a standard PC. The Windows™ based software was simple to operate and allowed the adsorption / desorption times, as well as whether liquid or headspace samples were to be run and the number of vials to be sampled to be down-loaded to the GC autosampler.

All SPME and GC-MS data acquisition was performed on Windows™ based software (with the exception of the Finnigan Incos 500 GC-MS system). A Varian GC Star Workstation (version 3.1) was used for the SPME data handling which allowed multiple channel input and complete data manipulation, including calibration. GC-MS data handling was performed using Hewlett Packard GC-MS Chemstation software (version C.02.03) which contained the NBS mass spectral library having over 75,000 entries. This was used, together with a self-prepared pesticide and PAH library, for the

METHODOLOGY RESULTS AND DISCUSSION

Chapter 5

Extraction of Organic Pollutants from an Aqueous Matrix

5.1 Extraction of Organic Pollutants from an Aqueous Matrix

There has been much emphasis placed on the affect the many variables in SFE have on extraction efficiency. However, if analytes which have been successfully extracted are not efficiently trapped, prior to analysis, there is little hope of a quantitative extraction. Despite this, commercial SFE instruments frequently do not have adequately designed collection devices capable of trapping the wide range of analytes extracted using SFE. This chapter discusses the modification of two such commercial collection units which were found to allow extracted analytes to escape *via* small solvent droplets formed during the depressurization of the supercritical fluid.

Once the collection efficiency of extracted analytes has been assessed using an inert matrix, it is useful to empirically determine the relative solubility of the target analytes in the supercritical fluid at the density chosen (pressure and temperature combination). This may be achieved by extracting the compounds from an inert matrix which does not affect the overall extraction efficiency as a "real" matrix would. The optimization of both extraction pressure and temperature for the removal of organochlorine pesticides from Celite is detailed further in this chapter by use of a simple factorial experimental design. The optimum conditions are then used to extract the same analytes direct form an aqueous matrix, an area where SFE is rarely used. The recoveries obtained from the direct extraction are compared to those from a solid-phase extraction - supercritical fluid extraction technique where the analytes are trapped onto a solid sorbent prior to elution with a supercritical fluid.

In addition, the direct extraction from water theme is continued when a method utilizing solid-phase microextraction for the removal of semi-volatile compounds (s-triazine herbicides) from water is discussed. This technique completely removes the need to use organic solvents during the sample preparation stage of the analysis.

5.2 Optimization of Both Supercritical Fluid Extraction and Collection of Organochlorine Pesticides from an Inert Matrix

In all analytical scale SFE it is essential that, once the analytes have been extracted, the SFE system is capable of efficiently trapping the analytes using any one of the techniques discussed in the SFE instrumental section. Perhaps the best way to determine collection efficiency is by extracting known concentrations of the target compounds from an inert matrix which acts purely as a support and does not affect the overall extraction. Once quantitative collection has been achieved, the SFE operating

conditions can be optimized from the same inert matrix which gives an indication of the solubility of the compounds at the pressure and temperature chosen. However, the role of a "real" matrix cannot be neglected and if quantitative extraction is to be obtained from real samples, the "optimum" conditions may require re-optimization.

Procedure

Collection Vessel Optimization

Carlo Erba Collection Vessel Initial experimentation was performed to determine the collection efficiency of the Carlo Erba SFE. Lindane (structure shown in appendix 1) was used in the study and was prepared as an acetone stock solution and "spot" spiked (10 μg) onto 0.2 g ± 0.01 g of Celite and the solvent allowed to evaporate at room temperature. The sample was then transferred to a 1.67 ml extraction cell and extracted with relatively high density CO_2 (0.883 g ml^{-1}), corresponding to operating conditions of 300 atm and 50 °C. The fixed restrictor was crimped to allow a CO_2 flow-rate (under these conditions) of approximately 2 ml min^{-1}, with the depressurized gas being bubbled through approximately 4 ml of hexane contained in the Carlo Erba collection vessel described in section 4.1(c). Since the Carlo Erba uses a fixed restrictor which does not allow the exact flow-rate to be controlled, the amount of CO_2 which was passed through the extraction cell was kept constant at 30 ml (monitored at the pump) which took approximately 15 minutes under dynamic extraction conditions. The sample was then transferred to a 10 ml graduated flask, made up to the mark with hexane to a final concentration of 1 μg ml^{-1} (for 100 % recovery) and 10 μg of hexabromobenzene added as an internal standard. The extract was analyzed by GC-ECD under the conditions described in section 4.6. A relatively large volume of CO_2 was passed through the sample (over 17 cell volumes swept) in order to produce a substantial amount of aerosol formation within the collection vessel and therefore determine whether poor collection efficiency was responsible for any loss in analyte recovery. Second extractions were carried out on the same sample, under identical extraction conditions, with the addition of 500 μl of methanol direct to the Celite as a modifier.

Modified Collection Vessel Identical operating conditions were utilized when using the modified extraction vessel, described in section 4.1(c). However, before the extraction was commenced, the C_{18} SPE cartridge used in conjunction with the collection vessel was pre-conditioned by passing 2-3 ml of methanol through the sorbent. Upon completion of the extraction, the cartridge was rinsed with

approximately 1 ml of methanol to remove any analytes adsorbed when depressurized CO_2 was passed through the sorbent packing.

Optimization of Pressure and Temperature Conditions

The effect of the pressure and temperature on extraction efficiency was investigated using a simple (2^2) factorial experimental design. Pressure constraints between 100 and 300 atm together with temperature constraints ranging between 50 and 80 °C were used to extract three different organochlorine pesticides (lindane, aldrin, and dieldrin, whose structures are shown in appendix 1) from Celite. Five separate experiments were then required to allow all of the possible pressure / temperature combinations (including one at the design centre) to be described, and are listed in table 5.1.

Trial Number	Pressure (atm)	Temperature (°C)
1	100 (-)	50 (-)
2	300 (+)	50 (-)
3	100 (-)	80 (+)
4	300 (+)	80 (+)
5	200 (0)	65 (0)

Table 5.1 Pressure / Temperature Combinations for the SFE Optimization Study from Celite.

The Celite samples were prepared, as before, by spiking with an acetone solution containing the three OCPs. Prior to each extraction, the fixed restrictor tip of the SFE was altered to ensure the CO_2 flow-rate remained around 2 ml min^{-1} regardless of the operating conditions. Experiments were then performed at the various pressure / temperature combinations whilst passing 30 ml of CO_2, with the extracts being collected in the modified collection vessel. As before, the hexane extracts were made up to 10 ml with hexane and hexabromobenzene added as an internal standard prior to analysis by GC-ECD.

Results and Discussion

Collection Vessel Optimization

The recoveries of lindane from spiked Celite, using the original Carlo Erba collection device, are shown in table 5.2.

Extraction No.	% Recovery
1	16.4
2	15.7
3	10.1
Average	14.1

Table 5.2 Extraction Recoveries for Lindane using the Original Carlo Erba Collection Unit.

The poor recovery obtained using the initial operating conditions cannot be explained by the insufficient solubility of lindane in supercritical CO_2 since many SFE publications successfully extract lindane and other OCPs using lower densities of CO_2.[116,117] In addition, the low extraction recovery is unlikely to be caused by strong adsorption of lindane to the sample matrix since a polar inert support was used. When a second methanol modified extraction was performed on the same samples, no detectable amount of lindane was found. This led to the assumption that the poor recovery was due, not to an inefficient extraction, but to poor collection efficiency using the relatively large volume Carlo Erba collection unit.

The modified collection unit, utilizing a C_{18} SPE cartridge was then evaluated under identical extraction conditions. The results are shown below in table 5.3.

Extraction No.	% Recovery
1	86.4
2	87.9
3	94.4
Average	89.6

Table 5.3 Extraction Recoveries for Lindane using the Modified Collection Unit.

Near quantitative results were obtained using the modified collection vessel, indicating that the aerosol formation caused by the depressurized CO_2 removed a significant concentration of extracted analyte from the collection vessel before analysis. Using the modified collection device, the analyte contained within the solvent aerosol droplets was trapped on the non-polar sorbent packing of the SPE column and rinsed back into the bulk solution after the extraction was completed. The addition of a second SPE column in series with the first did not improve the extraction recovery, implying that one column was sufficient to adsorb all of the analytes

contained within the aerosol. The successful collection unit was then used throughout the remainder of the SFE experimentation.

Optimization of Pressure and Temperature Conditions

The various experimental combinations, shown in table 5.1, were run in order in which they appear with the recoveries obtained listed in table 5.4. A typical GC-ECD chromatogram for a 1 $\mu g\ ml^{-1}$ standard used during the experimentation is shown in figure 5.1 overleaf.

Run Order	Pressure	Temperature		% Recovery Lindane	% Recovery Aldrin	% Recovery Dieldrin
1	-	-	y_1	49.9	85.2	90.8
2	+	-	y_2	85.3	95.7	95.8
3	-	+	y_3	41.1	92.5	71.7
4	+	+	y_4	84.1	88.7	81.7
5	0	0	y_5	75.9	86.0	88.5

Table 5.4 Extraction Recovery of OCPs from Celite in 2^2 Factorial Design.

The responses (y) to the various factor combinations in the 2^2 factorial design were used to calculate the main effects of the factors in the experiment. As discussed in chapter 3, the global effect of pressure (similarly for temperature) is defined as half the difference between the average of the responses at the high level (+) and the average of the responses on the low level (-). Also the interaction between pressure and temperature is defined as half the difference between the effects of temperature at the high and low pressure levels. Thus, the main and interaction effects were calculated following the equations shown in appendix 2. The results for all of the OCPs are given in table 5.5.

Pesticide	Main Effect Pressure (%)	Main Effect Temperature (%)	Interaction (%)
Lindane	19.6	-2.5	1.9
Aldrin	1.7	0.1	-3.6
Dieldrin	3.8	-8.3	1.3

Table 5.5 Main and Interaction Effects Calculated from the 2^2 Factorial Design.

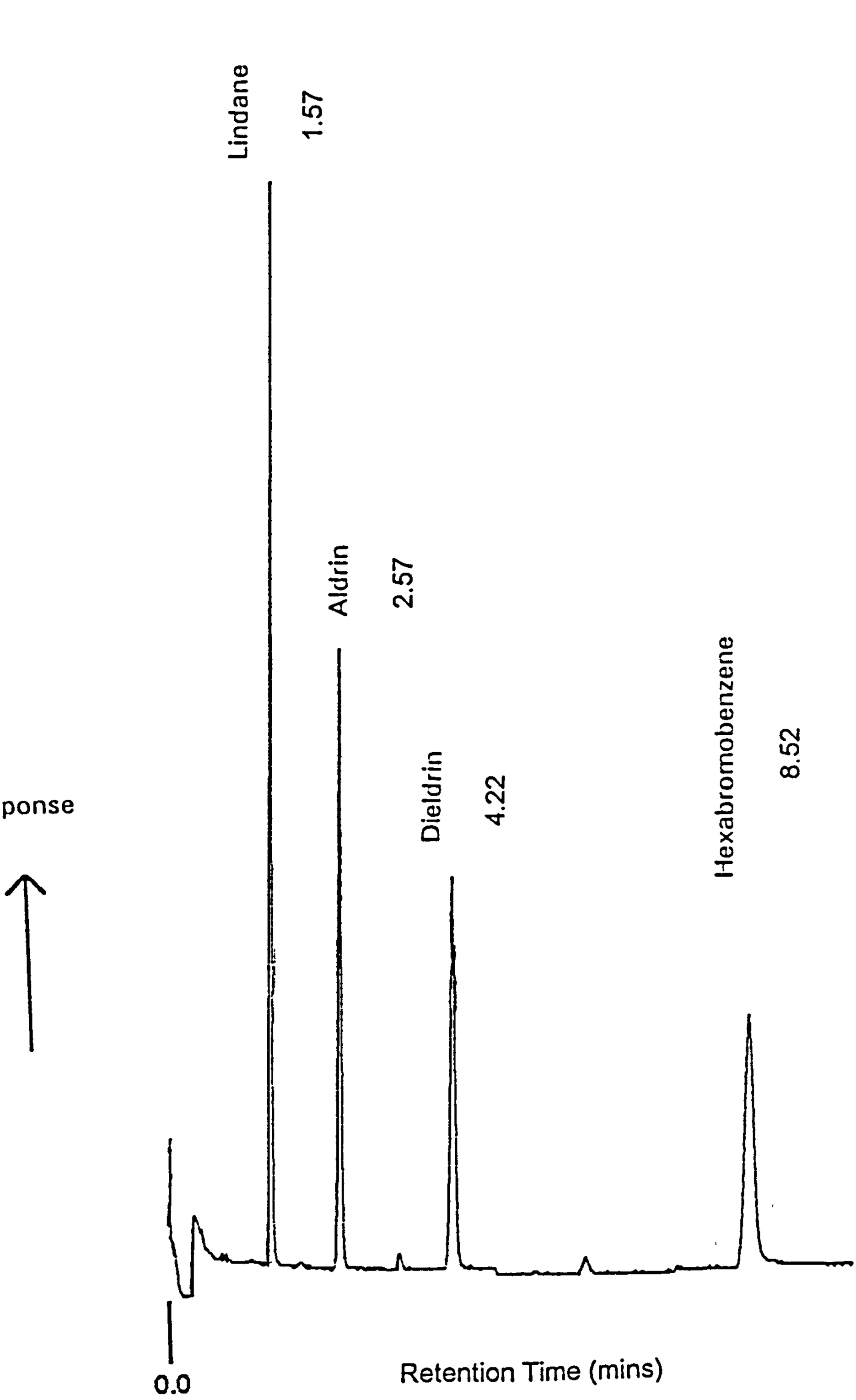


Figure 5.1 A Typical GC-ECD Chromatogram.

The magnitude of the main and interaction effects indicates the relative effect of pressure and temperature on extraction recovery. All of the effects are small (<5 %) with the exception of pressure for lindane and temperature for dieldrin. This trend can be observed in the raw recovery data where low recoveries are obtained for lindane at low pressures. The sign of the effect indicates whether increasing the size of the factor, (-) to (+), has an enhancing or detrimental effect on recovery. Therefore for pressure, all of the effects are positive with a large effect for lindane (19.6 %) indicating that pressure should be kept at a high value under optimum conditions. The opposite is true for temperature, with two out of three effects being negative with the largest for dieldrin being -8.3 %. This implies that the extraction temperature should be kept at the low factor value for optimum extraction conditions.

Overall, the factorial design showed that the optimum extraction conditions (within the set constraints) for extracting OCPs from an inert matrix were an extraction pressure of 300 atm together with a temperature of 50 °C (*i.e.* the highest density combination). These conditions were then used throughout the remainder of the experimental SFE described in chapter 5, although it should be noted that the time of extraction and the amount of CO_2 passed through the extraction cell may require alteration to obtain acceptable recoveries when extracting from a different matrix.

5.3 Direct Extraction of Organochlorine Pesticides from Water using Supercritical Fluid Extraction

There has been little research into the use of SFE to extract from aqueous samples, or even samples with a high moisture content, when compared with the wealth of publications involving SFE from solids. However, environmental analysis laboratories routinely screen thousands of water samples annually for a range of pesticides, far more than corresponding soil samples for the same analytes. Examples of the limited use of SFE to extract, both directly and in-directly, from water are given in section 2.3.1.3.

Procedure

Direct extraction of a water sample containing OCPs was evaluated using the "headspace" extraction cell described in section 4.1(d). The Jasco SFE (described in 4.1b) was used in the study because the large size of the 50 cm^3 cell prevented its use

in the relatively small Carlo Erba oven compartment. The optimized pressure and temperature conditions determined in the extractions from Celite were used in the experiments. The optimum operating conditions were used to extract lindane, aldrin and dieldrin (10 μg each in acetone) from 45 ml ± 1 ml of distilled water, leaving an approximate 5 ml headspace gap in the extraction cell (this was kept to a minimum to avoid overly long re-equilibration times after initial pressurization). The effect of flow-rate on recovery was investigated by extracting at 0.7, 1.0 and 1.5 ml min^{-1} (the Jasco SFE allows both pressure and flow-rate to be independently controlled). Extraction times were varied from 15 minutes to 2 hours, with the extracts being collected in hexane (5 ml) contained in the modified collection unit. Throughout all experimentation using the Jasco SFE, the collection vial was placed in an ice-bath to cool the collection solvent in an attempt to reduce the amount of aerosol formation caused by the "pulsing" of the CO_2 flow. This was found not to be necessary when using the Carlo Erba fixed restrictor system since the constant flow of depressurized CO_2 caused ice formation on the outside of the collection vial by the Joule-Thomson effect of the gaseous CO_2. In fact, if ice was used, the additional cooling during the extraction caused the CO_2 flow-rate to be greatly reduced because of problems with ice formation on the tip of the fixed restrictor.

The effect of adding sodium chloride (8 g ± 0.1 g) to the sample prior to extraction was also investigated. Salt is commonly used in liquid-liquid partitioning as it increases the ionic strength of the aqueous solution which in turn increases the relative hydrophobicity of the non-polar OCPs, aiding their removal from the matrix. The amount of salt used in the experiment corresponds to the sample : salt ratio used in the EPA protocol for extracting pesticides from drinking water.[335]

Results and Discussion

The recoveries of the organochlorine pesticides obtained at the three different flow-rates and after the addition of salt to the sample are shown numerically in appendix 3 through tables A3.1-A3.3. However, the overall trends are perhaps best visualized graphically in figure 5.2 illustrating the effect of CO_2 flow-rate on extraction efficiency and in figure 5.3 which illustrates the effect of increasing the ionic strength of the aqueous solution at constant flow-rate (1 ml min^{-1}).

As expected the recoveries increase with increasing extraction time as more CO_2 is allowed to pass dynamically through the cell. The number of cell volumes swept for

the three flow-rates studied (0.7, 1.0, 1.5 ml min^{-1}) at, for example, a typical extraction time of 60 minutes are 0.88, 1.25, and 1.88, respectively. Despite more than doubling the number of times the cell is swept with fresh fluid, *i.e.* at the flow-rates between 0.7 and 1.5 ml min^{-1}, figure 5.2 shows little difference in the amount extracted (with the exception of dieldrin whose recovery shows a slight reduction when using the lowest flow-rate).

The extraction curves are similar to that shown in figure 2.5 (SFE theory section) although the initial steep rise in the amount extracted is virtually absent in all cases. This type of extraction curve (figure 2.5) is normally observed with SFE from solid samples, where weakly bound analytes are initially rapidly removed. During this period, the solubility of the analytes in the supercritical fluid dictates the speed at which they are recovered. However, although solute diffusion through supercritical fluids is rapid, once analytes which are weakly bound to the surface of the matrix have been solubilized and removed, the rate of the latter part of the extraction is dependent upon the diffusion of the analytes through the sample matrix. The absence of the rise in figure 5.2 therefore implies that the extraction from water is limited by slow diffusion controlled kinetics. This is in agreement with the effect of increasing the flow-rate of the supercritical fluid which, if the extraction is solubility limited, will enhance the amount of solute recovered.[115] In the case of an aqueous matrix, no analyte is available for preferential extraction at the beginning of the SFE. In addition, analyte diffusion through the water matrix is slow and limits the partitioning of analytes in the diffusing CO_2. The steady rise in the extraction curves indicate that at even longer extraction times quantitative recoveries may have been obtained. However, the solubility of water in supercritical CO_2 (0.3 %) dictates that it would not be practical to extend the extraction time beyond two hours because of the increased amount of water carry-over observed in the hexane collection solvent at long extraction times. Therefore the maximum recovery obtained after 2 hours, for any analyte, is only approximately 70 %. This low recovery may be due to the excessively large cell volume used which, even after a two hour extraction at 1.5 ml min^{-1}, only allows 3.75 cell volumes to be swept with fresh CO_2. This is below the minimum number of cell volumes (four) suggested by Kane *et al.*[90] to allow sufficient contact between the sample and supercritical fluid. The amount extracted is similar for all of the compounds throughout the extraction profile, despite the variation in chemical structure. However, all of the analytes contain six sterically bulky chlorine atoms which may dictate their relative hydrophobicity in aqueous solution and lead to all of the molecules being extracted with relative equality.

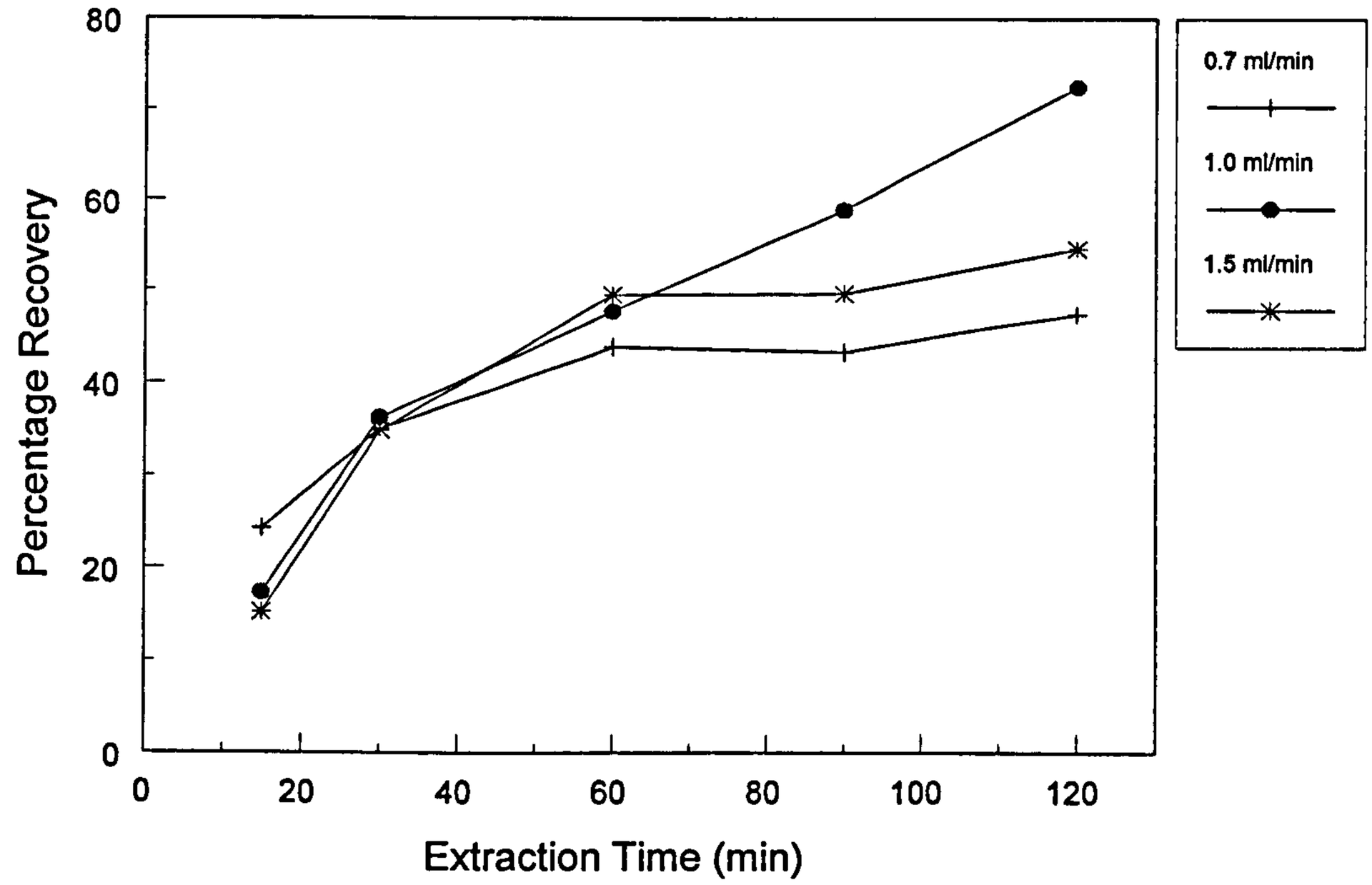


Figure 5.2a Percentage Recovery *versus* Extraction Time for Direct Extraction of Lindane from Water.

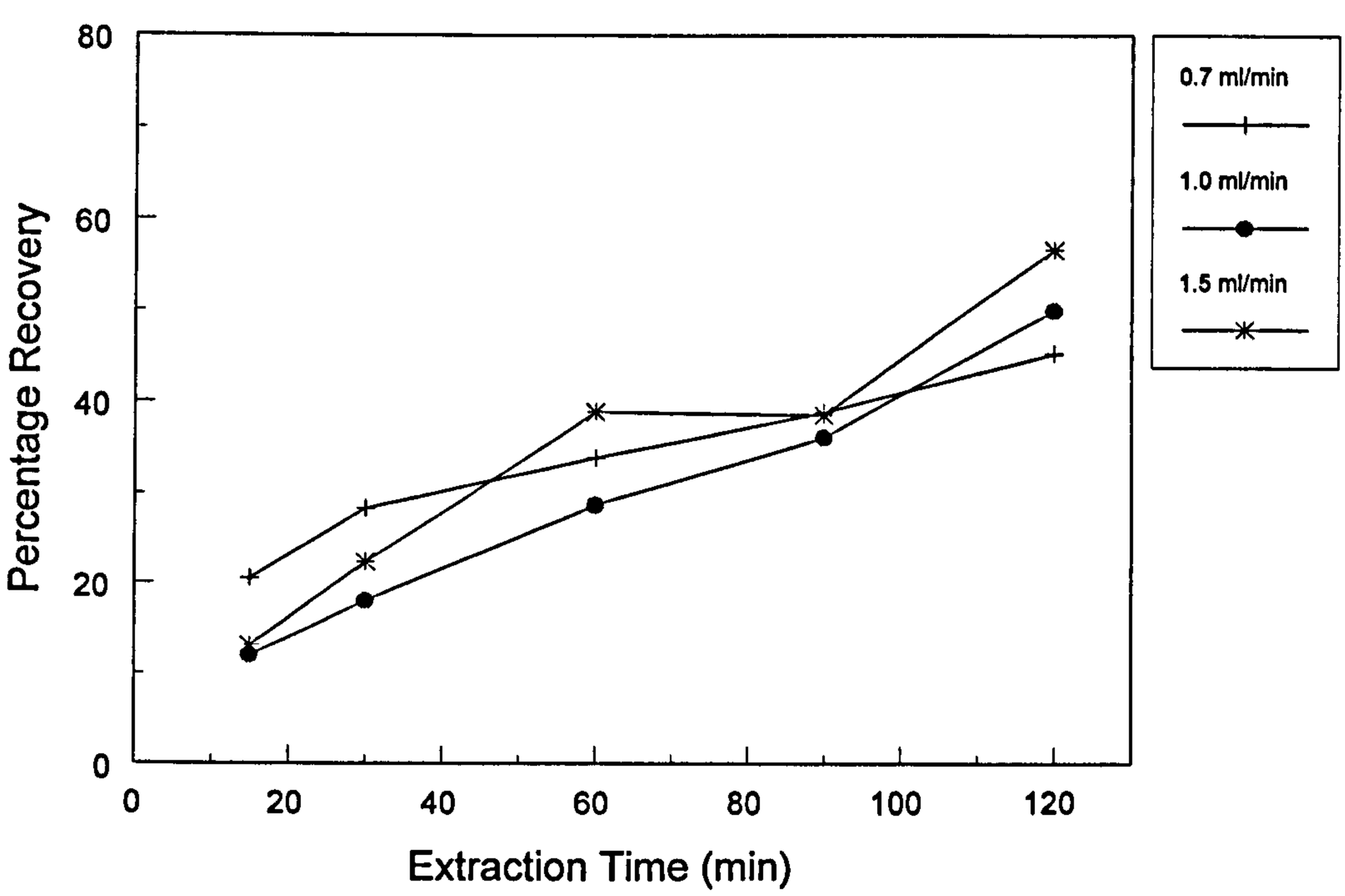


Figure 5.2b Percentage Recovery *versus* Extraction Time for Direct Extraction of Aldrin from Water.

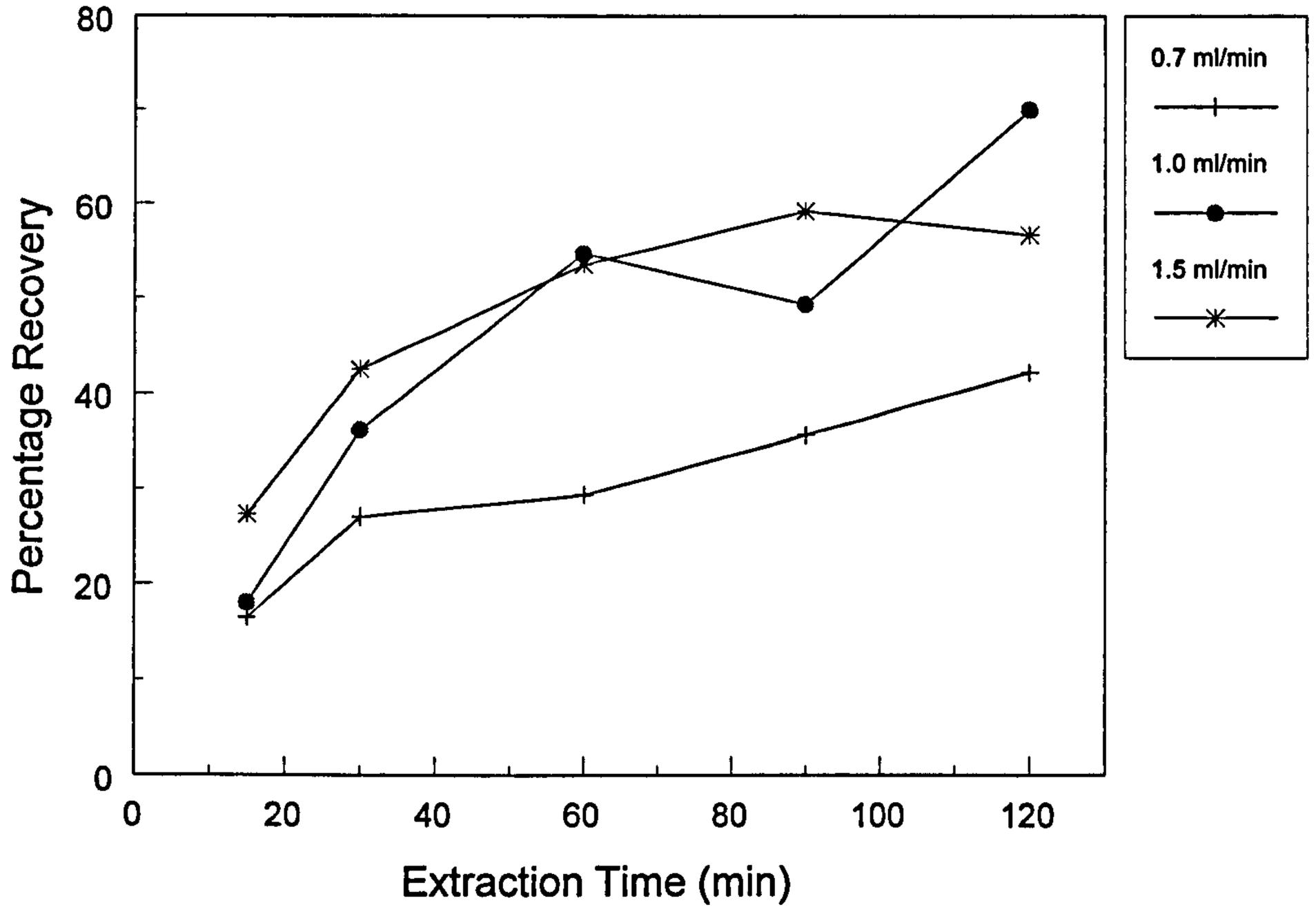


Figure 5.2c Percentage Recovery *versus* Extraction Time for Direct Extraction of Dieldrin from Water.

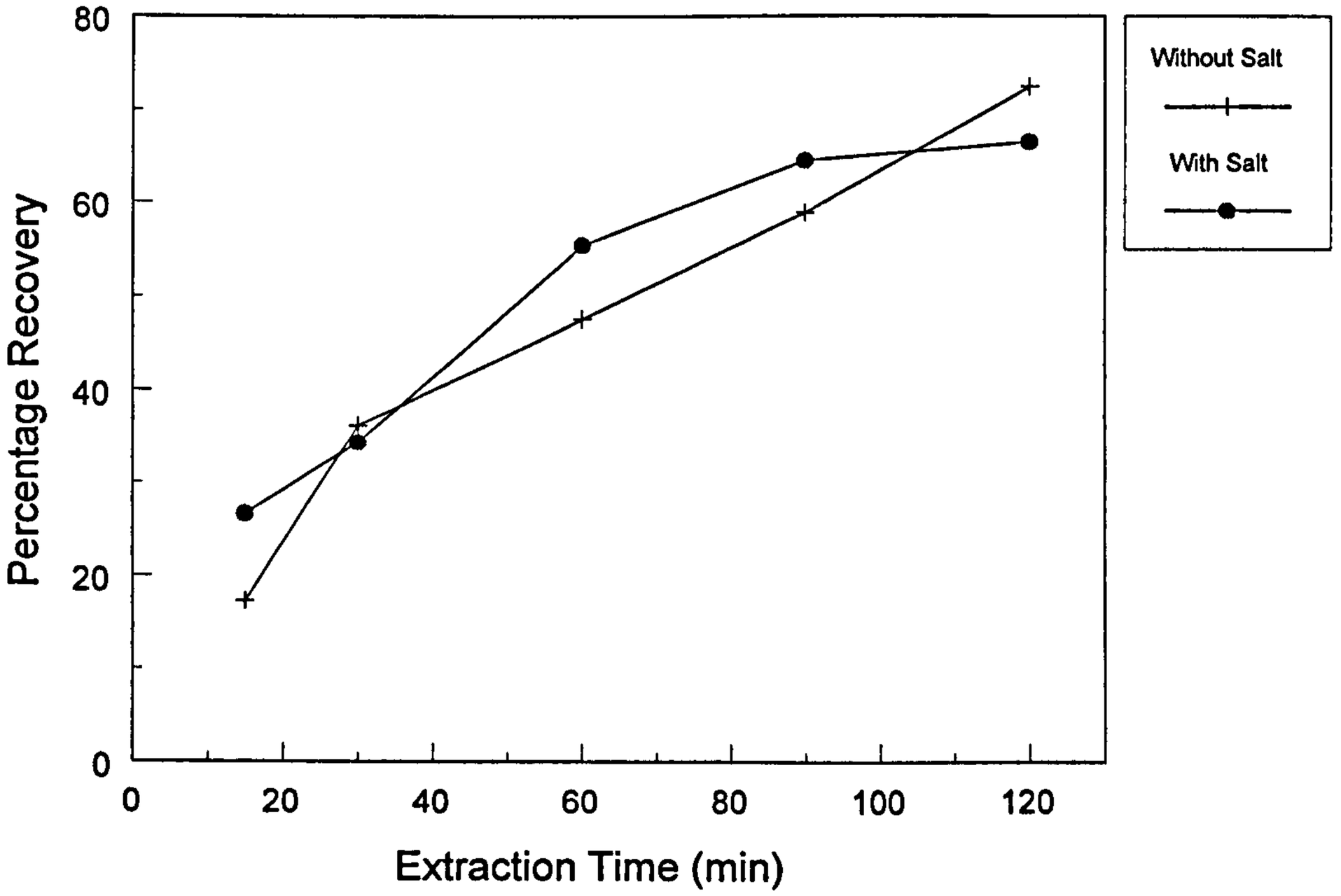


Figure 5.3a Effect of Salt on Lindane Percentage Recovery *versus* Extraction Time.

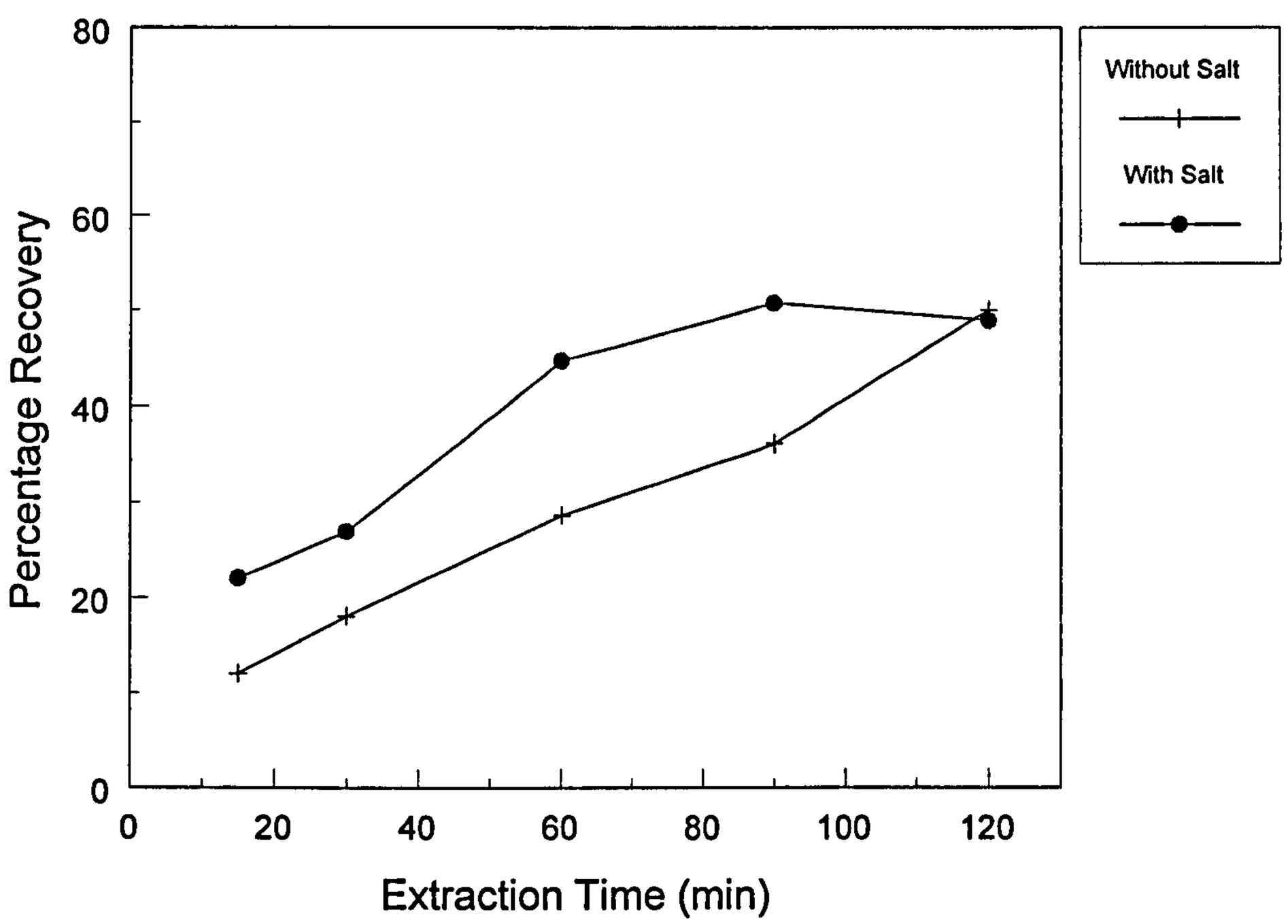


Figure 5.3b Effect of Salt on Aldrin Percentage Recovery *versus* Extraction Time.

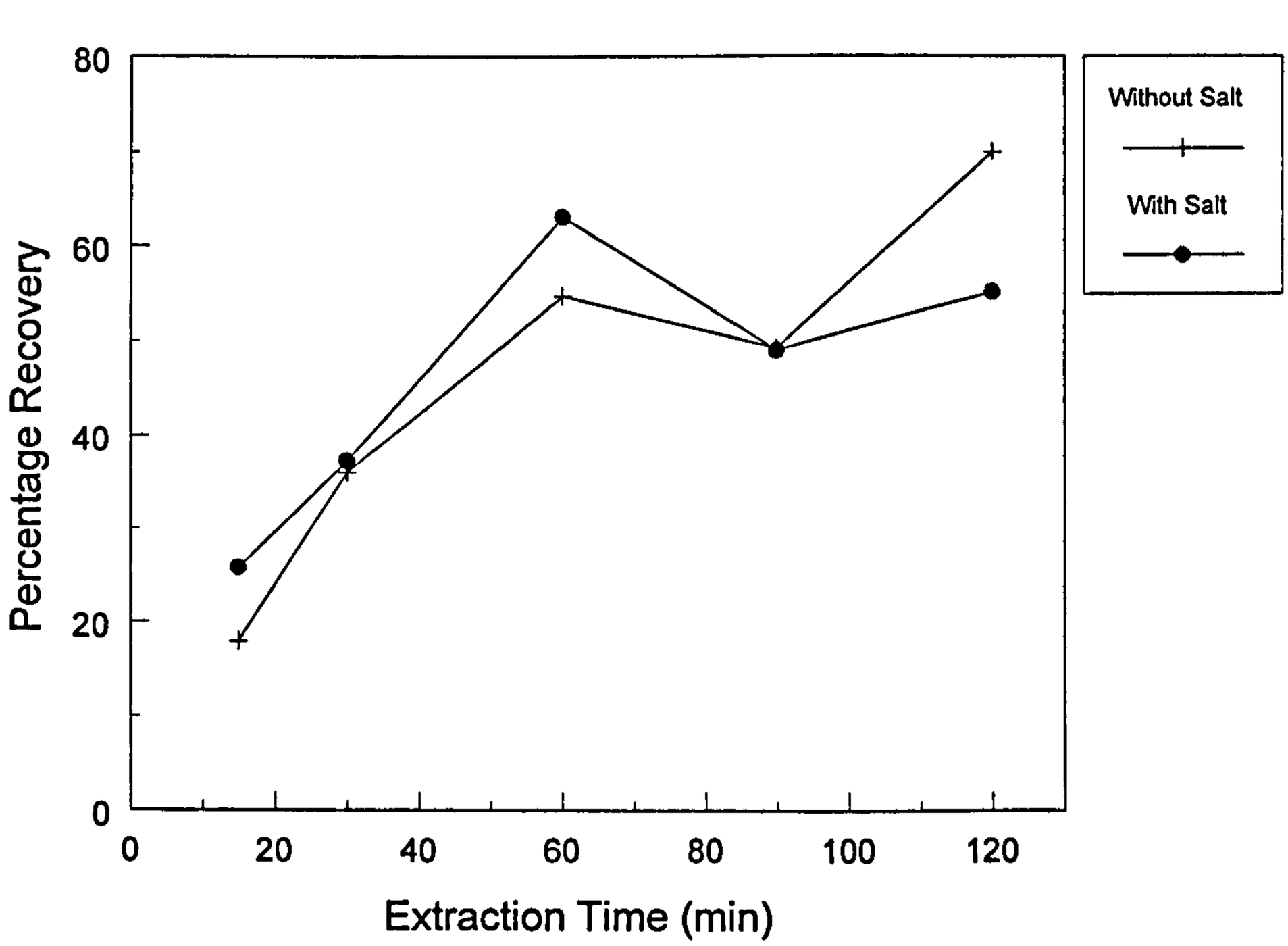


Figure 5.3c Effect of Salt on Dieldrin Percentage Recovery *versus* Extraction Time.

Although the effect of "salting out" is well known and is frequently used to assist liquid-liquid partitioning, the results reported in figures 5.3(a-c) indicate that the addition of sodium chloride to the sample has no significant effect on the overall recovery of the OCPs. This may be due to the much increased water carry-over observed when salt is added to the sample (even at short extraction times) causing problems in analyzing the OCPs using GC-ECD.[336] It therefore appears impractical to use salt to enhance the extraction efficiency of OCPs by supercritical CO_2.

To assess the errors in the extraction, a repeatability study was performed on a fifteen minute extraction at 300 atm, 50 °C and 1 ml min^{-1}. The percent relative standard deviation for lindane, aldrin, and dieldrin was found to be 6.7, 7.3, and 8.2 %, respectively (n = 5). The raw data for the study is shown in table A3.4 in appendix 3. These results indicate that the majority of the differences observed in figures 5.2(a-c) and 5.3(a-c) may be due to simple experimental error associated with the technique. This error is likely to increase at longer extraction times.

5.4 Solid-Phase Extraction-Supercritical Fluid Extraction Approach to Extraction of Pesticides from Water

By far the most common technique for extracting analytes from water using supercritical fluids has been by adsorbing the analytes on a solid support prior to supercritical fluid elution with CO_2. This technique has been further enhanced, in recent years, by the introduction of solid-phase extraction disks since after filtration they can be easily inserted into a standard SFE extraction cell. This SPE-SFE approach to aqueous extraction was used to remove lindane, aldrin, and dieldrin from spiked water samples in an attempt to improve their overall recoveries when compared to those obtained using direct SFE extraction.

Procedure

The details of the disk extraction procedure used are common throughout this section as well as sections 6.2 and 6.3 and are shown below.

In all studies involving the extraction of pollutants from aqueous samples, the samples used were fortified in the laboratory at a known concentration due to the difficulty in obtaining "real" aqueous samples containing known amounts of pollutants. A stock

solution containing the pesticides (or herbicides) of interest was prepared in a water soluble solvent (acetone or methanol) and a known volume was spiked directly into a 200 ml ± 2 ml distilled water sample. The fortification level of the pesticides was dependent on the sensitivity of the analytical technique which was used for the subsequent analysis of the extracts. The spiking level varied between 10 µg for OCPs with detection by GC-ECD (this section) and herbicides by HPLC (section 6.3), to 100 µg for OCPs by GC-MS (6.3) and finally to 200 µg for both OCPs and OPPs by GC-MS (section 6.2). Once the water samples had been fortified, they were pre-treated before extraction disk filtration by adding 5 ml ± 0.5 ml of methanol and adjusting the pH to less than 2 with concentrated hydrochloric acid.

A C_{18} Empore disk was then placed in a Millipore filtration assembly and pre-conditioned by activating with 10 ml ± 0.5 ml of methanol for 3 minutes and passing air through under vacuum for 1 minute. A further 5 ml ± 0.5 ml of methanol was added and allowed to soak for another 3 minutes followed by 10 ml ± 0.5 ml of distilled water and the sample, which was filtered under vacuum in around 5 minutes. During this stage, care was taken not to allow the disk to become dry. After all of the sample had been passed, the disk was air dried under vacuum for 10 minutes and subsequently placed in a warm oven (45 °C) for a further 20 minutes to ensure complete dryness (the non-volatile nature of the OCPs ensured that no analyte would be lost during the drying stage). This was found necessary to prevent fixed restrictor plugging due to ice formation which severely reduced the CO_2 flow-rate when using the Carlo Erba SFE system. The disk, containing the adsorbed analytes, was now ready for elution with supercritical CO_2 (although methanol was used as a solvent in initial studies involving the extraction of herbicides from water, in section 6.3).

The SPE disk was then rolled using tweezers and placed in a 10 ml SFE extraction cell. The optimum pressure and temperature obtained from the Celite experimentation was used throughout the study. A 30 minute static extraction was then carried out to allow the supercritical CO_2 to "soak" into the disk and was followed by a dynamic extraction where two lots of 30 ml of CO_2 were passed through the cell at a flow-rate of approximately 2 ml min^{-1}. The hexane extracts were combined and analyzed by GC-ECD as before.

Results and Discussion

As mentioned previously, the optimized SFE conditions obtained from extraction of the target analytes from an inert matrix may require alteration when extracting from a

"real" sample. In the case of the SPE-SFE experiment, an additional static extraction period was deemed necessary to allow efficient removal of the OCPs. Also, the amount of CO_2 dynamically passed through the cell was doubled to account for the large increase in cell volume (10 ml compared to 1.67 ml used in the optimization procedure), although the second aliquot of CO_2 was later found to only elute 2-3 % of the total. The recoveries of the OCPs are shown in table A3.5 (appendix 3). Overall average recoveries and percentage relative standard deviations were, 77.0 % (12.4 %), 98.5 % (6.7 %), and 91.6 % (9.5 %), for lindane, aldrin, and dieldrin, respectively (based on four repeat extractions, with RSD shown in brackets).

It is seen that quantitative recoveries are possible for aldrin and dieldrin using a combined SPE-SFE method. However, the recovery for lindane is less than expected although this may by due to experimental error since the RSD for lindane is high (subsequent extractions in the selectivity study between OCPs and OPPs show quantitative recovery of lindane is possible). Overall, the method compares favourably to that of direct extraction with around 20 % higher recovery obtained. In addition, the intermediate drying stage of the SPE disk prevents any water from being carried-over to the collection solvent therefore allowing problem free gas chromatographic analysis. As well as the advantage of higher recoveries, the overall SPE-SFE procedure takes approximately 100 minutes per sample (including all filtration, drying, and supercritical elution stages), around 20 minutes faster than the long two hour extraction required to obtain only 70 % recovery by direct SFE extraction.

In summary, conventional collection devices supplied with two commercial instruments have been shown to ineffectively trap organochlorine pesticides extracted with supercritical carbon dioxide. A modified collection assembly incorporating a solid-phase extraction cartridge has been used to quantitatively collect the target analytes and will be used throughout all other SFE experimentation. Once the collection difficulties had been overcome, a simple experimental design was used to elucidate the optimum density required to extract OCPs from Celite. The operating conditions were then used in the extraction of OCPs from an aqueous matrix which has been achieved by two different methods. The techniques are simple and on the whole are equivalent to, or marginally faster than conventional liquid-liquid partitioning. Solid-phase extraction disks have been shown to be efficient at trapping OCPs and give quantitative results with supercritical CO_2. Direct extraction from water was not affected by the addition of salt to the matrix or by changes in the supercritical fluid flow-rate which indicates that the extraction process is limited by

slow diffusion through the aqueous sample. Despite less than quantitative recovery, the method can potentially be used as a qualitative screening technique.

5.5 Solid-Phase Microextraction of s-Triazine Herbicides from Water

The continuing large-scale use of s-triazine herbicides has necessitated their constant monitoring in drinking water supplies. Conventional low-solvent sample preparative methods involve the sorption of the herbicides from water samples onto a solid material prior to elution with an organic solvent(s). Unfortunately, solid-phase extraction does suffer from some disadvantages, which have been discussed in section 2.3.2.1. In addition, they are usually disposed of after a single extraction, and still require some solvent usage. Solid-phase microextraction (SPME) has been investigated as an alternative to conventional SPE for the extraction and analysis of four s-triazines from water (simazine, atrazine, propazine, and trietazine, structures shown in appendix 1), using a fully-automated SPME system.

Procedure

Due to the semi-volatile nature of the s-triazine herbicides, liquid sampling was used throughout the experimentation as opposed to headspace sampling often successfully used to extract volatile compounds. Aliquots (1.2 ml ± 0.05 ml) of all aqueous solutions (standards prepared in acetone and used to spike the aqueous samples at known concentrations) were injected into 2 ml autosampler vials to ensure the fibre was fully immersed in the solution (whilst adsorbing) and to prevent sample-to-sample variation. In addition, all adsorptions were carried out at ambient temperature (approximately 23 °C) and were unstirred.

An initial study involving the manual injection of an acetone solution containing the four herbicides was used to determine a temperature programme to allow the adequate separation of the four compounds. The retention times obtained were then used to confirm the identity of the peaks desorbed from the 7 μm fibre.

A Experimentation Involving the 7 μm Fibre

Initial experimentation involved the use of a 7 μm poly(dimethylsiloxane) coated fibre which was specifically designed for the extraction of semi-volatile analytes. The relatively thin coating on the fibre enabled the phase to be chemically bonded to the silica support, allowing a maximum desorption temperature of 320 °C. This high operating temperature is used to allow the desorption of high boiling organic compounds. As with the 100 μm fibre, the 7 μm fibre was first desorbed at its maximum operating temperature, with the injector split vent open, for a minimum of three hours and then repeatedly desorbed at the same temperature whilst running the GC oven programme, until no peaks were detected.

Effect of Column Focusing Temperature

The effect of the column focusing temperature was investigated to determine which optimum GC oven temperature allowed the efficient focusing of the herbicides desorbed in the GC split/splitless injection port. A range of initial oven temperatures were chosen between 40 and 100 °C, at 10 °C intervals, since it was not possible to cool the column below 40 °C in a short period of time without oven cryogenics. The effect on peak shape and area was noted for a 10 minute adsorption of a 1 μg ml^{-1} solution of the four herbicides studied, using the 7 μm fibre. The fibre was then desorbed for 15 minutes at 270 °C (half way between the minimum and maximum fibre operating temperatures) with the column kept at a constant temperature within the range chosen. The usual GC temperature programme (described in section 4.7) was used to elute the compounds from the column regardless of the initial starting temperature.

Effect of Desorption Temperature

The effect of desorption temperature on the 7 μm fibre was determined over the working range of the fibre (220-320 °C). The fibre was used to adsorb a 1 μg ml^{-1} solution for 10 minutes before a 15 minute desorption at the temperature studied. The lowest column focusing temperature studied (40 °C) was used to focus the desorbed analytes. At the end of each extraction, a blank desorption was carried out at the maximum operating temperature of 320 °C to show any carry-over at lower desorption temperatures.

B Experimentation Involving the 100 μm Fibre

Despite the intended use of the 100 μm fibre for the extraction of volatile analytes, the fibre was investigated as an alternative to the thinner-coated fibre for the extraction of the semi-volatile herbicides. The fibre has one distinct advantage over the thinner fibre in that the amount of analyte adsorbed from solution is directly proportional to the amount of phase present on the fibre. This in turn affects the method sensitivity and therefore its overall applicability to environmental analysis.

Comparison Between the 7 μm and the 100 μm Fibre

The 7 μm fibre was replaced with a 100 μm fibre designed for use with volatile organic compounds. The additional phase on the thicker fibre is incapable of being chemically bonded to the silica support, which means that lower operating temperatures must be used to prevent phase bleed during desorption. The actual working temperature of the 100 μm fibre is between 120 and 220 °C.

A comparison between the adsorption capacity of the two fibres was achieved by extracting a 1 μg ml^{-1} solution using a 5 minute and 15 minute adsorption period for the 100 and 7 μm fibres, respectively (a shorter adsorption time was used so as not to overload the thicker fibre). Both fibres were then desorbed for 15 minutes at 220 °C and 250 °C, respectively, and the resulting chromatograms compared.

The appearance of the two fibres was also compared using scanning electron microscopy.

Comparison Between a Manual Injection and a SPME Extraction using the 100 μm Fibre

A manual injection of a 1 μg ml^{-1} solution of the four s-triazines studied was compared with a HPLC grade water sample containing the same concentration, extracted using the 100 μm fibre. An acetone solution was manually injected into the split/splitless injector at 250 °C with the split vent closed. The split vent was subsequently re-opened after 0.75 minutes. This was compared with a SPME extraction using the freshly blanked 100 μm fibre which was adsorbed for 5 minutes in a 1 μg ml^{-1} aqueous solution. After the adsorption was completed, the fibre was desorbed for 15 minutes in the GC injector at 220 °C. During the desorption stage the column temperature was maintained at 40 °C which allowed the desorbed analytes to be re-focused at the front end of the analytical column. The resulting chromatograms were then compared.

A comparison between a new (un-used) 100 μm fibre, a fibre which had been used approximately 20 times, and an old fibre (> 150 extractions) was undertaken using scanning electron microscopy to determine the effect of repeated use on the fibre phase coating. All three fibres were compared together on a relatively low magnification (30X) and individually at higher magnification (100X).

Effect of Desorption Temperature on the 100 μm Fibre

The most efficient desorption temperature was determined by maintaining a constant adsorption time of 5 minutes and using a 0.3 μg ml^{-1} solution so as to reduce the size of the peak areas obtained with the thicker fibre. This ensured that accurate integration was always possible. After the adsorption period was completed, the fibre was inserted into the injector, which was maintained at various temperatures ranging from 220 °C to 120 °C, for 15 minutes (the temperature range recommended to be used with the 100 μm fibre). After each extraction of the 0.3 μg ml^{-1} solution, the injector temperature was increased back to 220 °C and a blank desorption undertaken to ensure no carry-over occurred.

Effect of Adsorption Time

The 100 μm fibre was used to investigate the effect of adsorption time on the peak areas of the four herbicides. A 1 μg ml^{-1} solution of the four analytes was used as a test mixture and the fibre was adsorbed over a range of times between 30 seconds to 15 minutes. After each adsorption the fibre was desorbed at 220 °C for 15 minutes.

Investigation of the Dynamic Range of the 100 μm Fibre

SPME is predominately an equilibrium based technique and for quantitation, calibration standards must be taken through the extraction procedure. A short adsorption time of 5 minutes and desorption time of 15 minutes at 220 °C were then used to study the dynamic range of the 100 μm fibre. A series of aqueous working solutions containing the herbicides were made from the stock standards in ranges between 1.0-0.1 μg ml^{-1}, 0.1-0.01 μg ml^{-1}, and 0.01-0.001 μg ml^{-1}. Due to time constraints, each set of working standards were run on consecutive days.

Multiple Extractions and Analysis of Low Concentration Solutions

Within Europe the EEC has set limits for individual pesticides and herbicides in drinking water of 0.1 μg l^{-1}. It is therefore essential for any screening method to be able to detect at this level. From the previous study using the 100 μm fibre it was obvious that it would be impossible to detect a 0.1 μg l^{-1} solution of s-triazine herbicides in a single extraction. Multiple adsorptions were therefore carried out on

the same sample vial and desorbed in the injector. However, instead of following each desorption with a temperature programme to elute the analytes, the herbicides were "stacked" at the front of the column which was maintained at the focusing temperature (40 °C).

Multiple extractions obviously take considerable time and to reduce this the optimum desorption time was briefly investigated. A 0.5 μg ml^{-1} solution of the four herbicides was placed in the carousel and adsorbed for 3 minutes using the 100 μm fibre. The fibre was then desorbed at 220 °C for the usual 15 minutes. This was compared with an identical adsorption but with a desorption time of only 5 minutes. Following the shorter desorption time a blank was run to determine any carry-over of analyte.

The multiple extraction technique was used to extract a 0.1 μg l^{-1} solution of the herbicides. A 10 minute adsorption followed by a 5 minute desorption, was repeated ten times from a single solution and "stacked" at the front of the GC column. The whole procedure (15 minute cycle x 10 repeats) was repeated three times.

Results and Discussion

C Experimentation Involving the 7 μm Fibre

Effect of Column Focusing Temperature

Initial experimentation involved the investigation of the GC column temperature required to allow adequate focusing of desorbed analytes. The results of the column focusing study are shown in full in appendix 3 (table A3.6). Overall it is concluded that the initial GC oven temperature has no significant effect on peak area (or peak height) with relative standard deviations (between the different temperatures) for simazine, atrazine, propazine, and trietazine of 39 %, 11 %, 12 %, and 6 %, respectively. The high RSD for simazine was attributed to its significantly lower response. In addition, the peak width at half height ($W_{1/2}$) was manually determined and was found to show no significant variation over the temperature range studied, indicating that the peak shape remained sharp and constant. It was therefore decided to use an initial column focusing temperature of 40 °C since there appeared to be no real benefit in using higher temperatures.

Effect of Desorption Temperature

The effect of the desorption temperature of the 7 μm fibre on the peak area obtained from a 1 μg ml^{-1} solution is shown in table A3.7 in appendix 3 and shown graphically in figure 5.4.

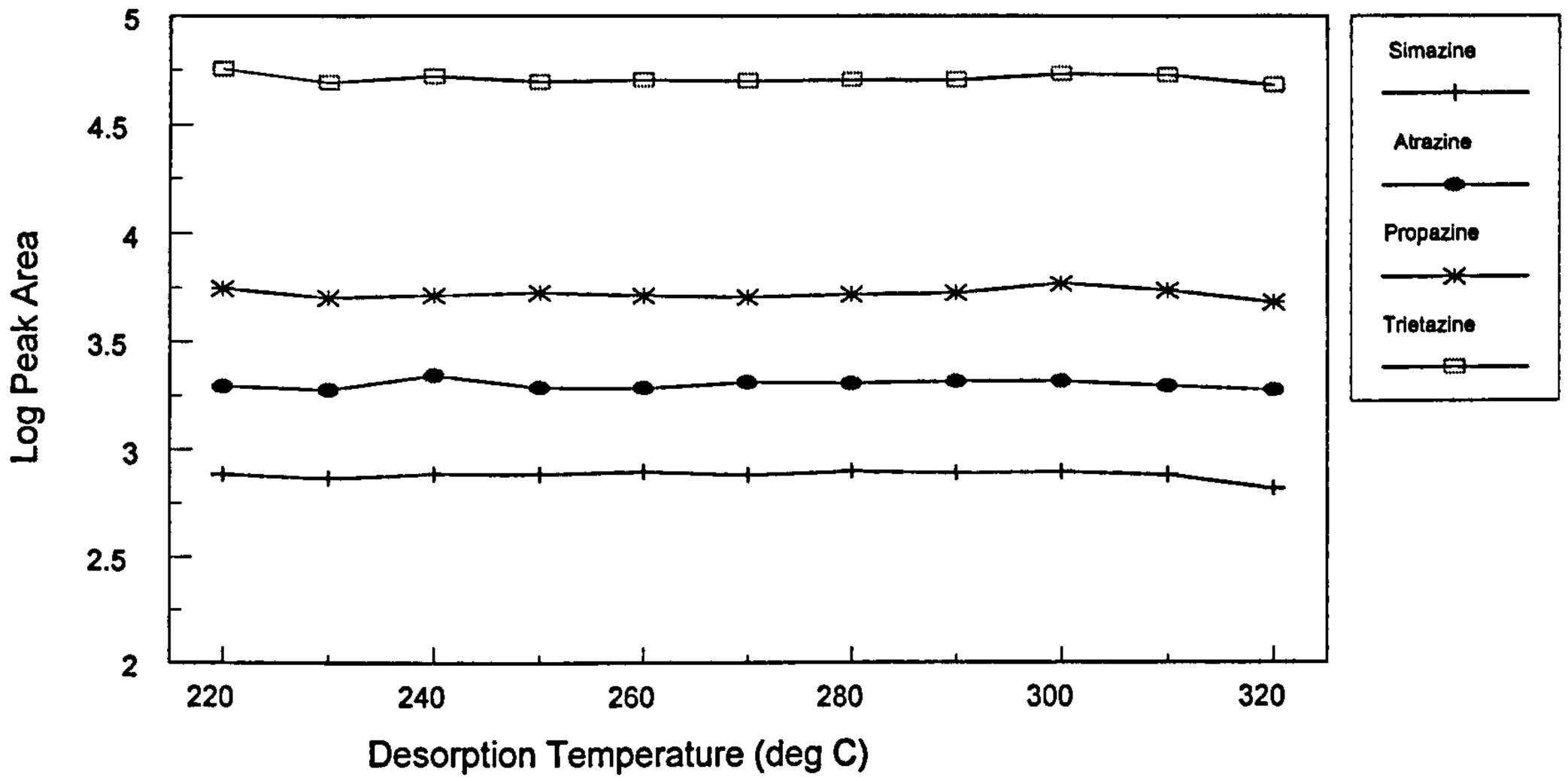


Figure 5.4 Effect of Desorption Temperature on Peak Area for 7 μm Fibre.

Conditions: 1.0 μg ml^{-1} solution, 10 minute adsorption time, 15 minute desorption time.

No difference in the peak area over the temperature range studied (220-320 °C) was noted. Additionally, no analyte carry-over was observed using a blank desorption at 320 °C after any of the extractions. Therefore, it was concluded that all of the adsorbed analytes were capable of being removed at the lowest desorption temperature using the 7 μm fibre.

The results of this study indicate that despite the relatively low maximum operating temperature of the 100 μm fibre, the thicker fibre may be of use in extracting the semi-volatile herbicides. This would be advantageous since the thicker fibre allows a greater amount of analyte to be adsorbed over a fixed adsorption period.

D Experimentation Involving the 100 μm Fibre

Comparison Between the 7 μm and the 100 μm Fibre

The difference in the amount adsorbed between the 100 μm and the 7 μm fibres is illustrated by the chromatograms in figure 5.5. The 100 μm fibre, despite having only one third the adsorption time of the 7 μm fibre, clearly allows a far greater

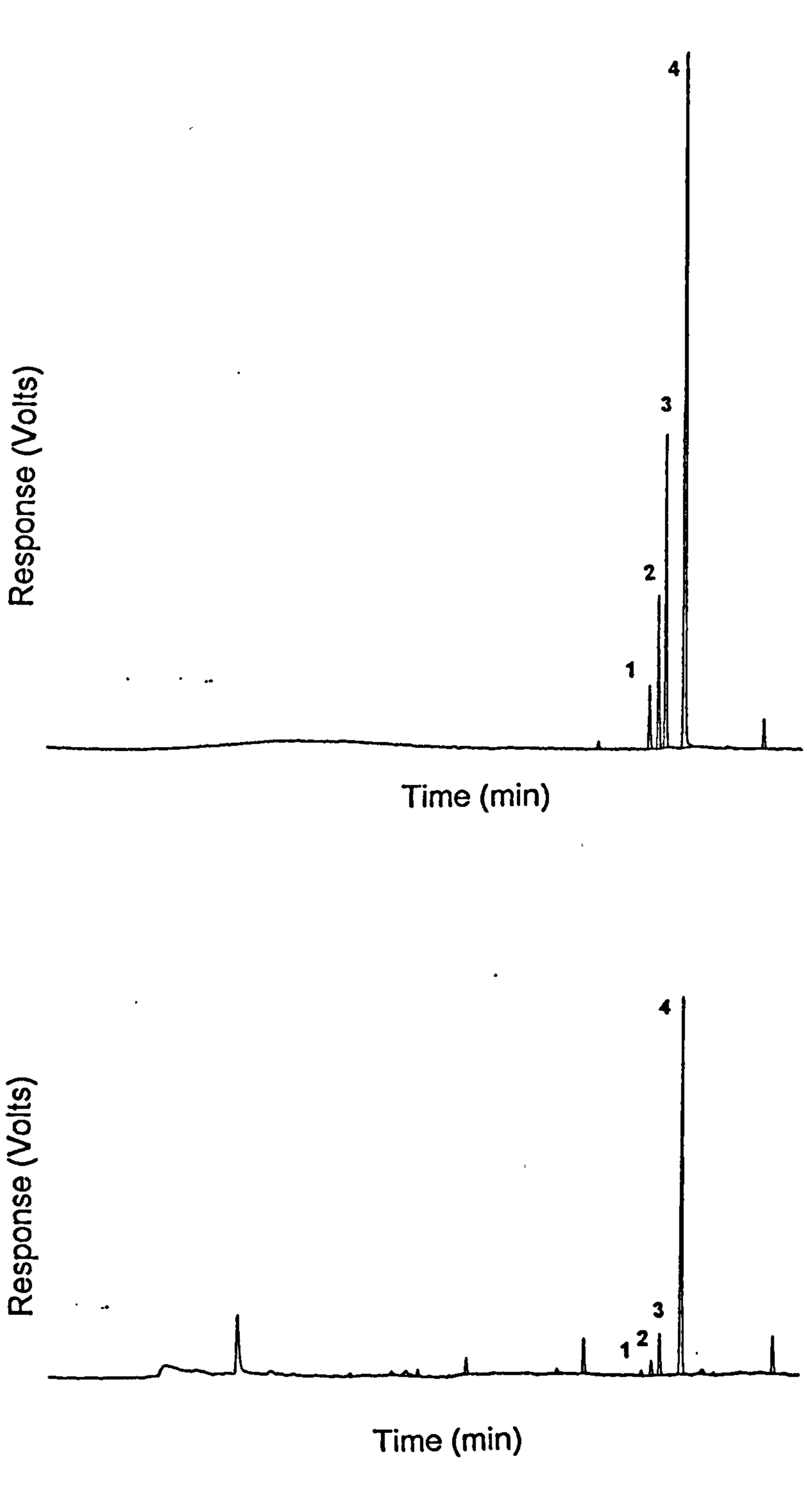


Figure 5.5 Comparison Between a 1 μg ml^{-1} Extraction Using a 100 μm Fibre (Upper) and a 7 μm Fibre (Lower).

Elution Order: (1) Simazine, (2) Atrazine, (3) Propazine, (4) Trietazine

Conditions: 100 μm fibre adsorbed for 5 minutes, 7 μm fibre adsorbed for 15 minutes; both desorbed for 15 minutes at 220 °C and 250 °C respectively.

concentration of analytes to be adsorbed when compared to the thinner fibre, as well as showing no reduction in the quality of the peak shape.

The difference in the film thickness' of the two fibres is perhaps best shown by use of a scanning electron microscopy photograph depicting the fibres (figure 5.6).

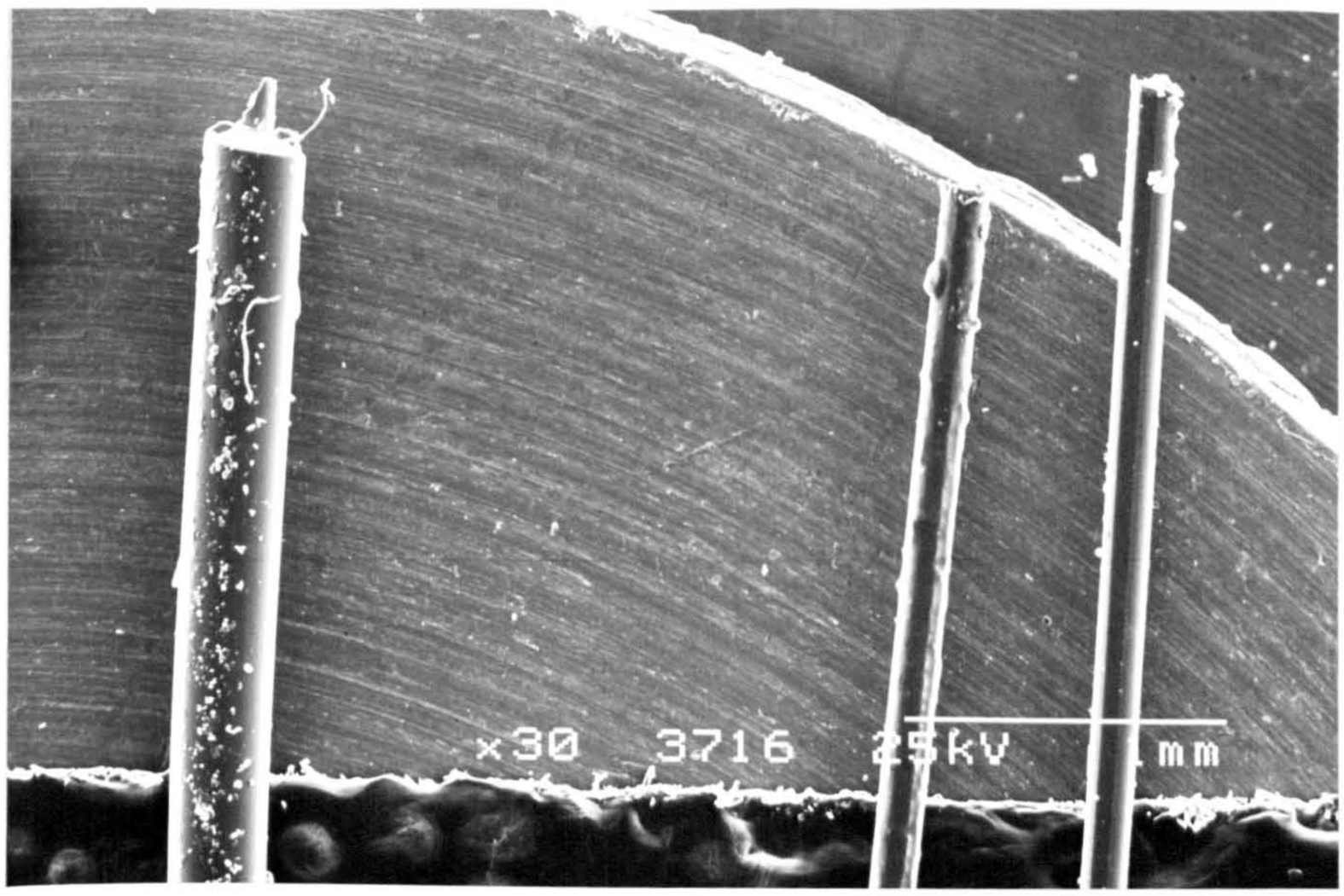


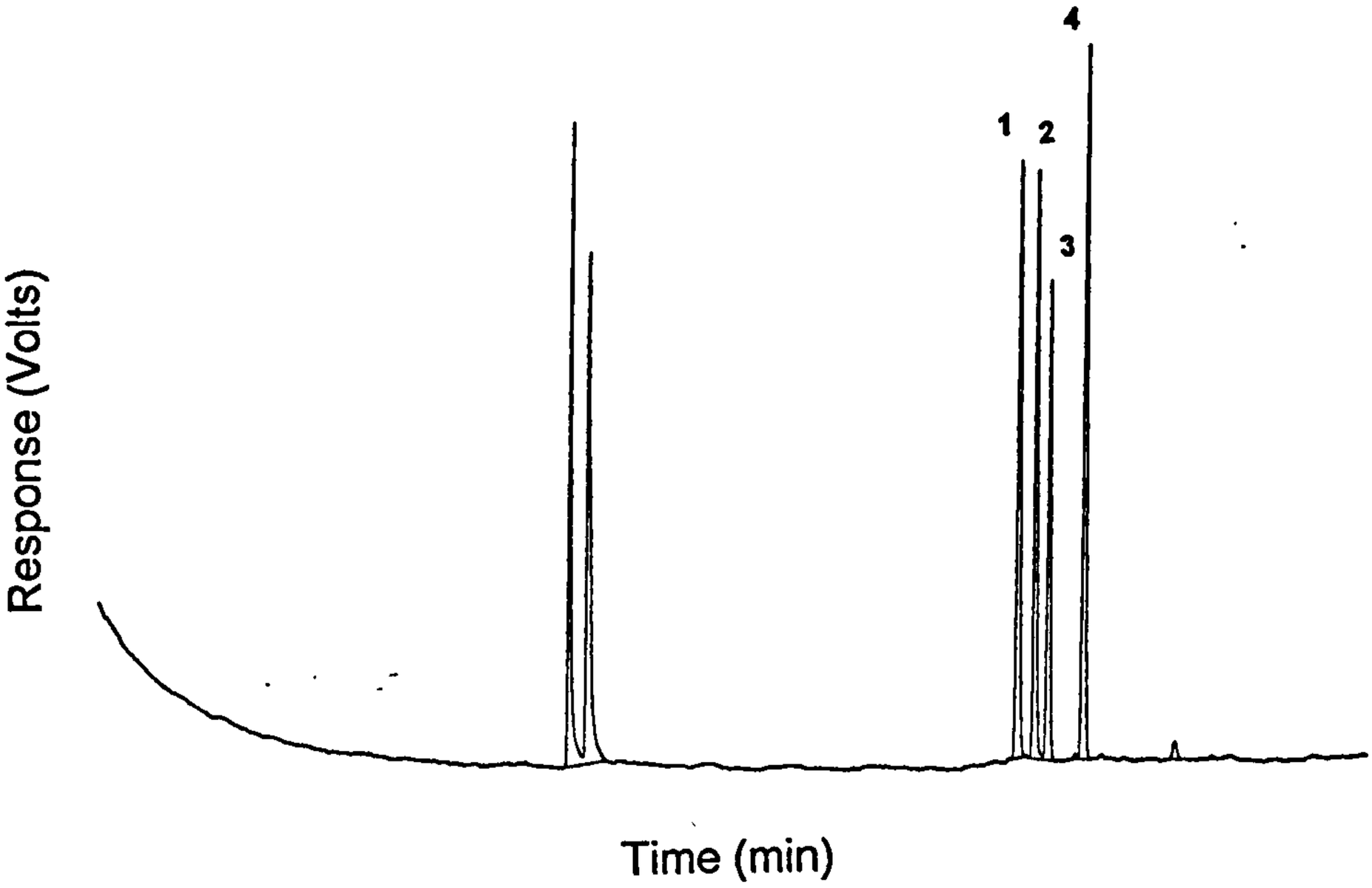
Figure 5.6 Scanning Electron Microscopy Photograph of the 100 μm and the 7 μm Fibres.
New 100 μm fibre (left), new 7 μm fibre (right), old 7 μm fibre (middle).

It was therefore decided to use the 100 μm fibre exclusively in all subsequent experimentation because of its greater loading capacity when compared to the thinner 7 μm fibre.

Comparison Between a Manual Injection and a SPME Extraction using the 100 μm Fibre

The two chromatograms resulting from the manual injection of the four herbicides and the SPME of the analytes using the 100 μm fibre are shown in figure 5.7.

It is obvious from the chromatograms that there is no degradation of peak shape during a SPME extraction and that peak width does remain constant with no tailing observed. This indicates that a column temperature of 40 °C during desorption is sufficiently low to successfully focus all of the herbicides. Also the retention times of the four peaks in the extraction exactly match those in the manual injection indicating that the peaks shown in the second chromatogram are actually the s-triazine herbicides



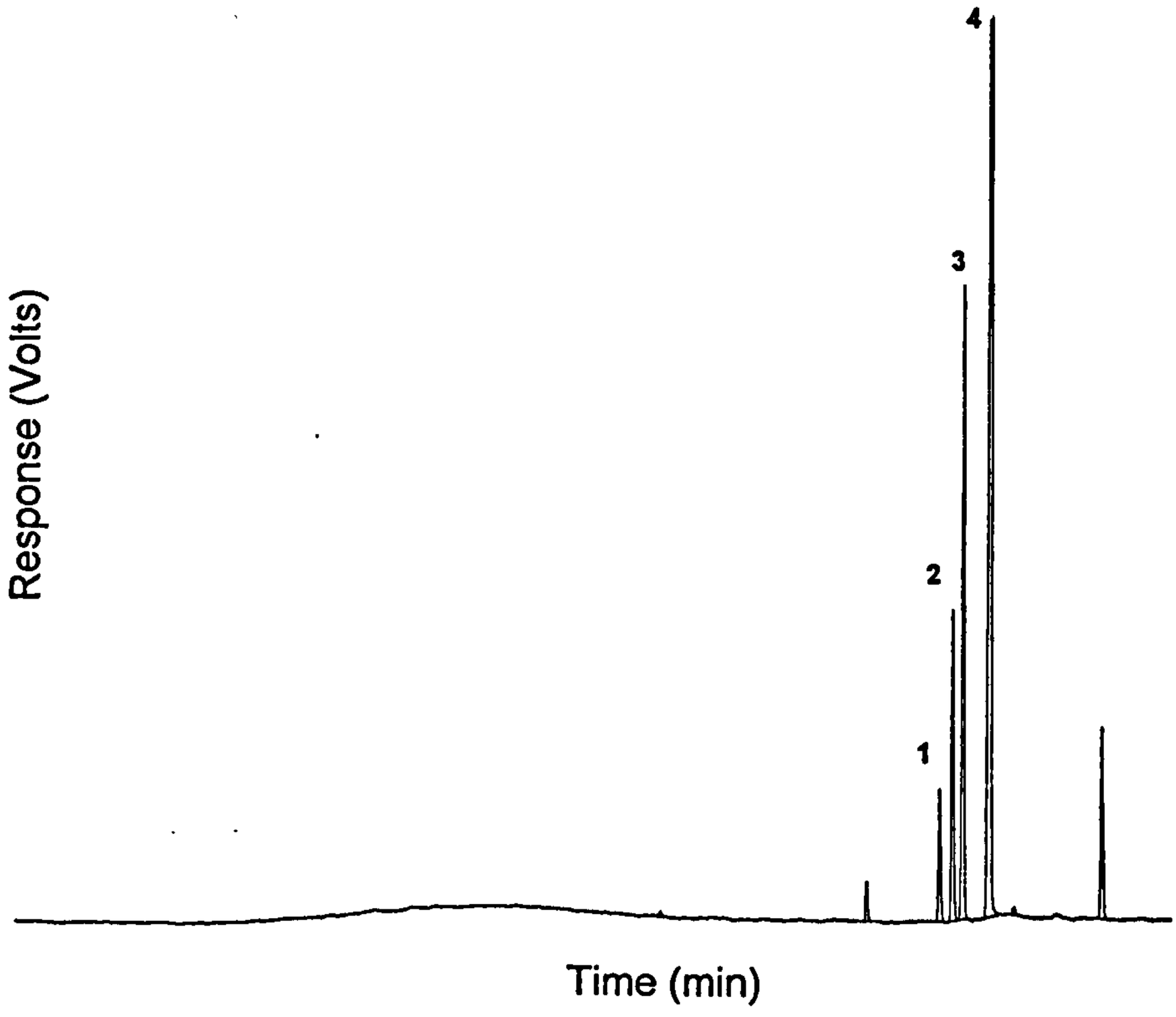


Figure 5.7 Comparison Between a Manual Injection (Upper) and a SPME Extraction Using a 100 μm Fibre (Lower).

Elution Order: As before.

Conditions: 1 μg ml^{-1} solution injected in acetone; SPME extraction, 5 minute adsorption followed by a 15 minute desorption at 220 °C.

of interest. However, one obvious difference between the two traces is the large discrepancy between the peak heights of the four herbicides when compared with the manual injection. This may be explained if the octanol / water partition coefficients (Log P) of the herbicides are considered as they indicate the relative hydrophobicity of the individual analytes. The Log P values for simazine, atrazine, propazine and trietazine are 1.96, 2.21, 2.91 and 3.07, respectively.[47] Therefore simazine is the least hydrophobic of the herbicides and so on. The extraction efficiency and thus the sensitivity of SPME depends on the analyte affinity towards the fibres sorbent coating (*i.e.* the partition coefficient between the analyte and the fibre) which in turn is dependent on the hydrophobicity of the individual analytes. The Log P values therefore explain why the relative peak size of four herbicides are always observed regardless of other operating conditions.

A comparison between a new 100 μm fibre (middle), a fibre which has been pre-conditioned (right) and an old fibre that has been used approximately 150 times (left) is shown in figure 5.8a. The picture was obtained using scanning electron microscopy and represents a 30 X magnification. The fibre which has undergone pre-conditioning is smoother than the new fibre indicating that some of the coating not strongly bound to the silica support has been thermally removed. When the old fibre is examined, the smooth nature compared to the other relatively new fibres is clearly seen. Also, the coating has begun to be mechanically removed (by continually being inserted and withdrawn from the sample vial and hot injector) from the tip of the fibre, exposing the silica support. The tips of the three fibres can be seen in greater detail in figures 5.8b to 5.8d where they are shown at 100 X magnification. Although some of the phase had been removed from the old fibre, no deterioration in overall performance was observed indicating that SPME fibres, even when being used for liquid sampling, are capable of being re-used around 100-200 times without serious deterioration in extraction ability.

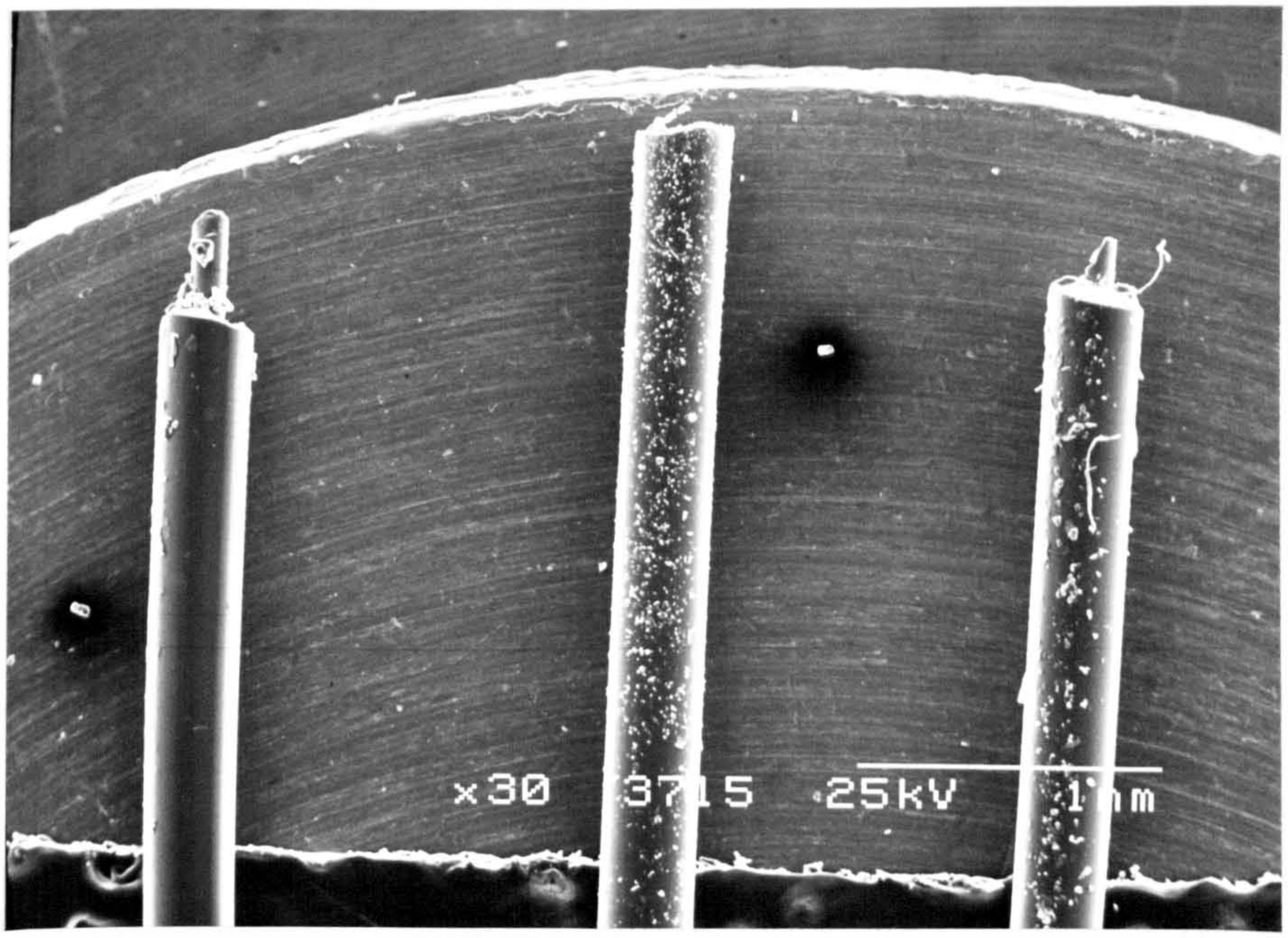


Figure 5.8a Comparison Between a New 100 μm Fibre (middle), a 100 μm fibre Used Approximately 20 times (right) and an Old Fibre (Used Around 150 times, left) at 30X Magnification.

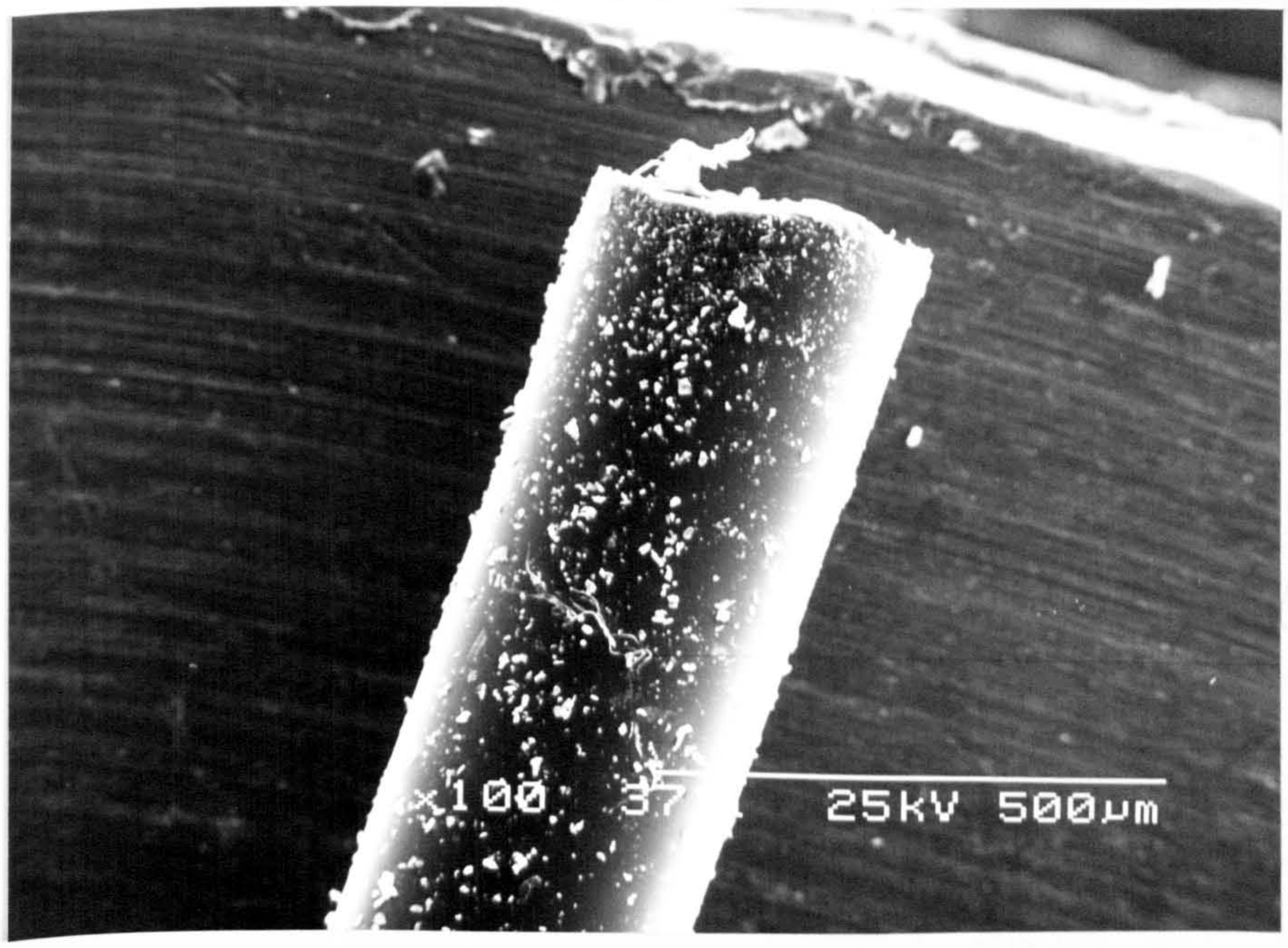


Figure 5.8b The Tip of a New 100 μm Fibre (at 100X Magnification).

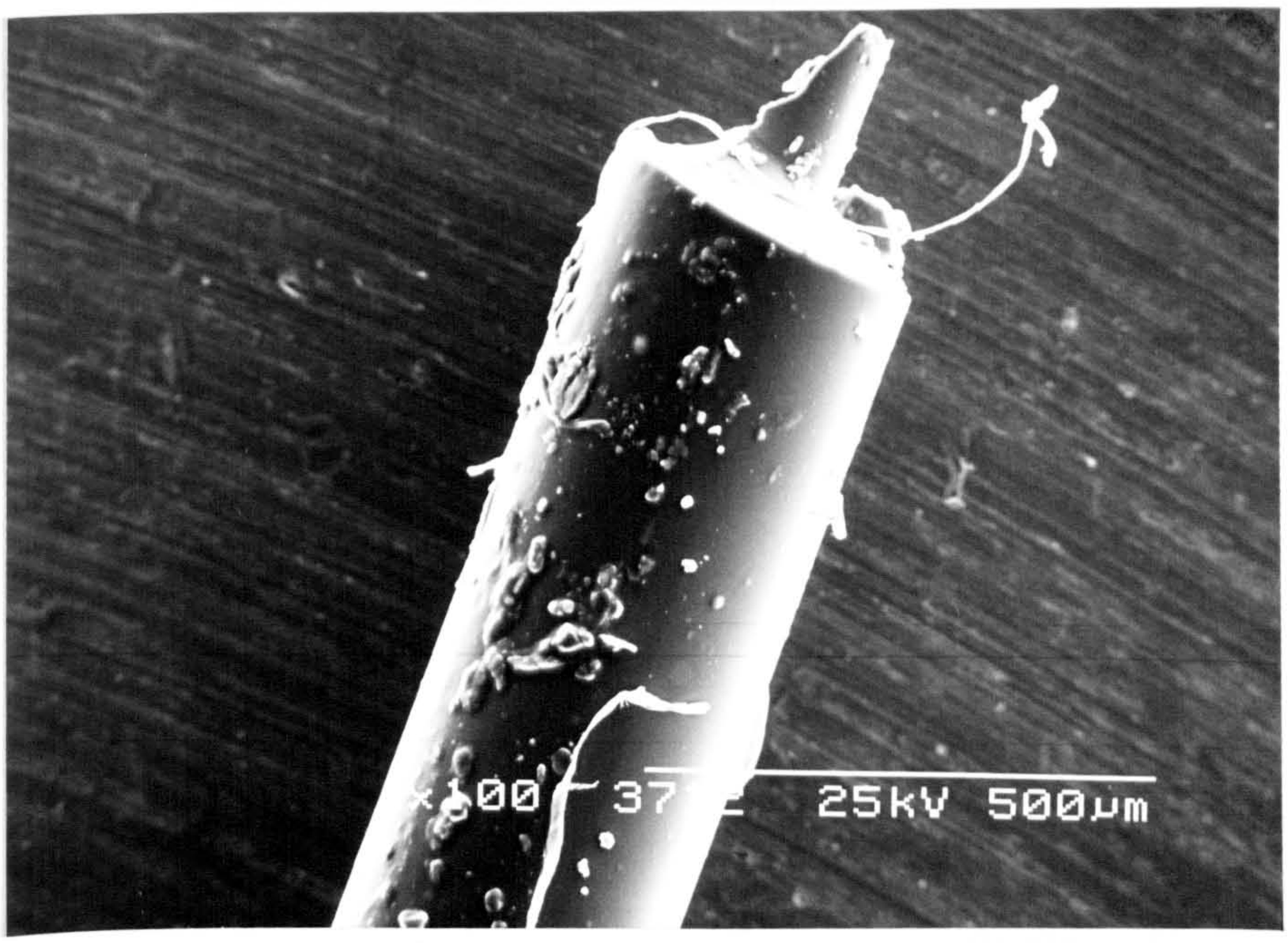


Figure 5.8c The Tip of a 100 µm Fibre Used Approximately 20 times (at 100X Magnification)

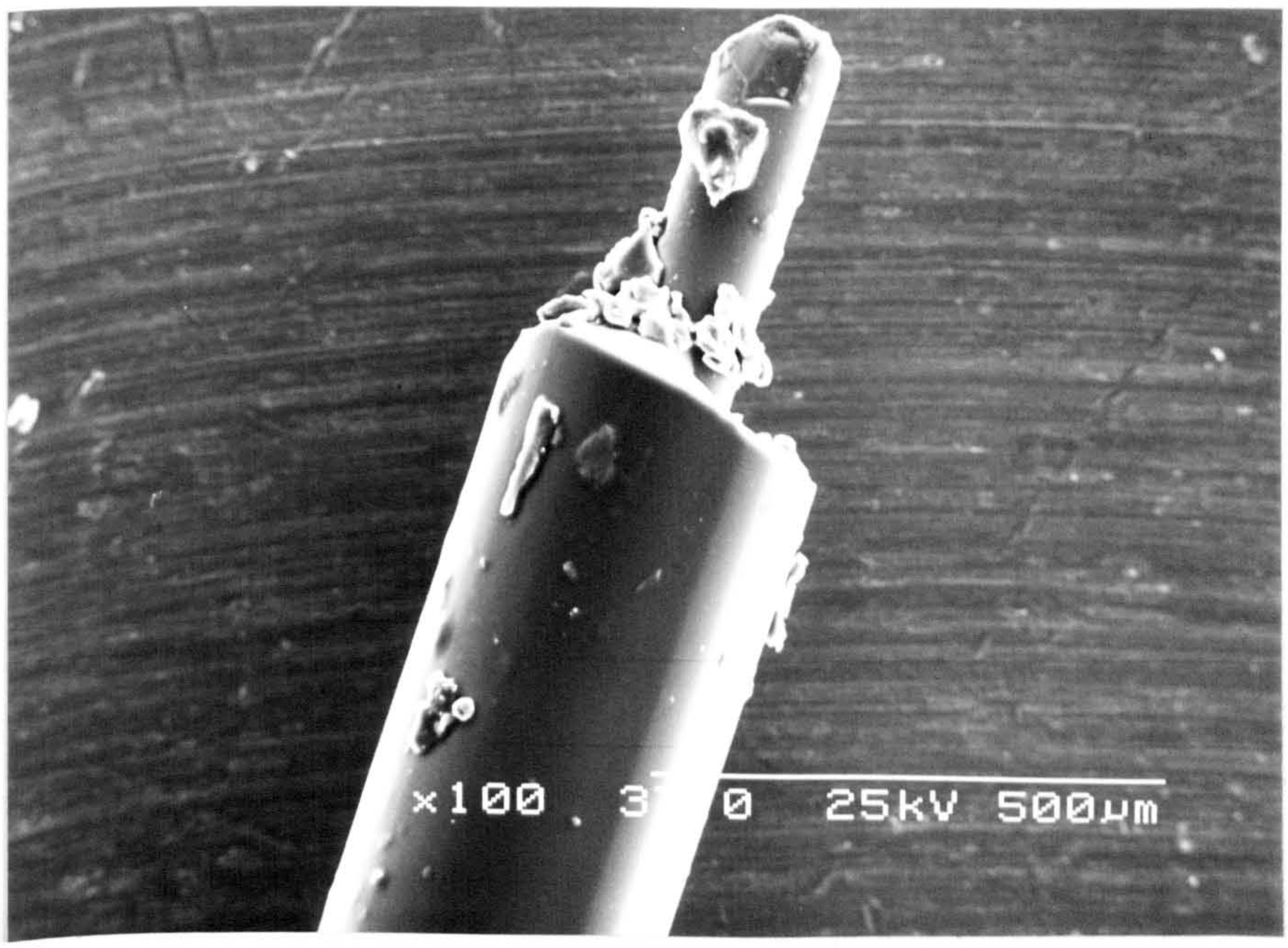


Figure 5.8d The Tip of an Old 100 µm Fibre Used Around 150 times (at 100X Magnification).

Effect of Desorption Temperature on the 100 μm Fibre

The results of altering the desorption temperature of the 100 μm fibre are shown in appendix 3 (table A3.8), however, are perhaps best illustrated in figure 5.9.

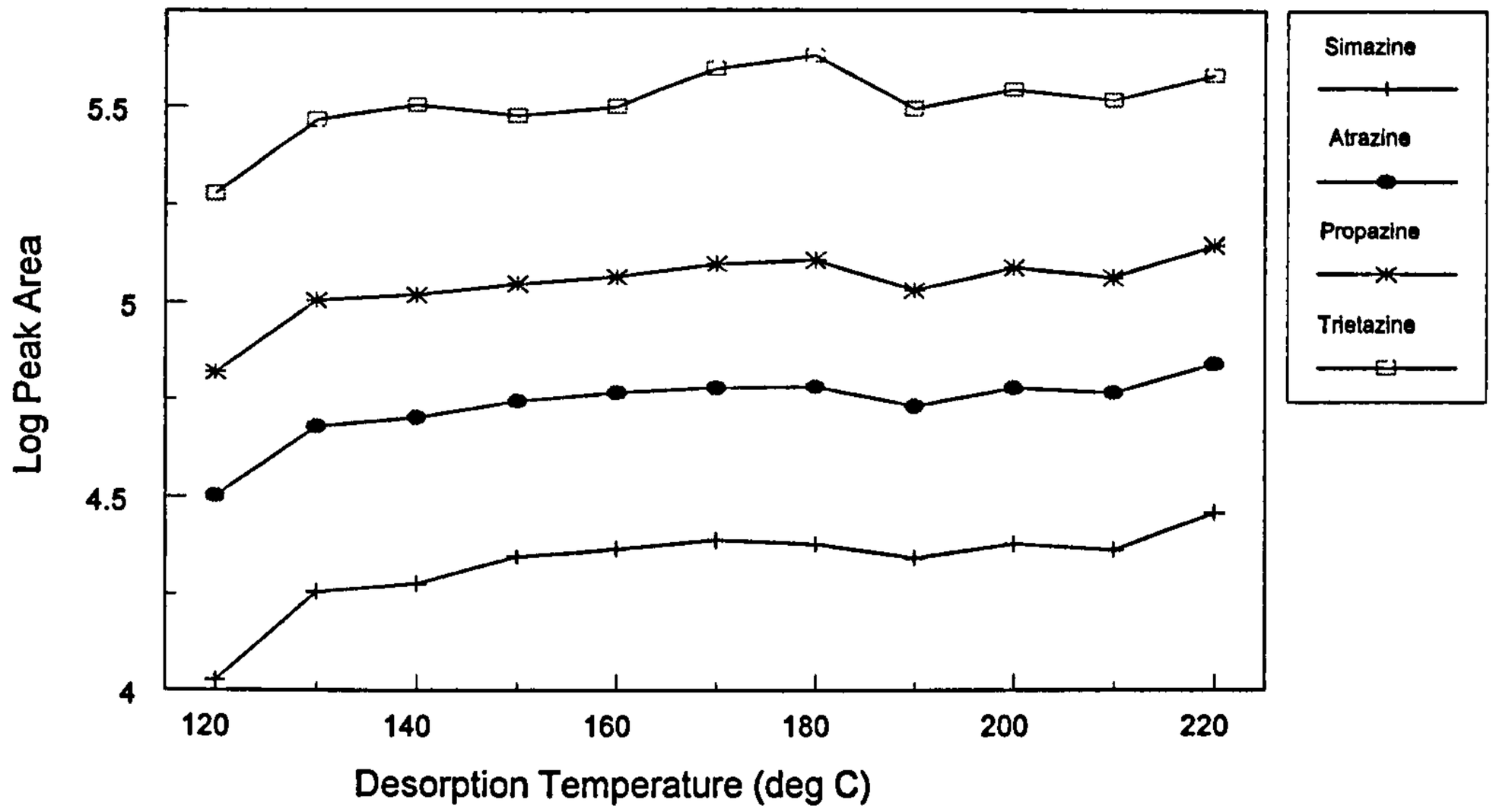


Figure 5.9 Effect of Desorption Temperature on Peak Area for 100 μm Fibre.

Conditions: 0.3 μg ml^{-1} solution, 5 minute adsorption time, 15 minute desorption time; blank desorption carried out at 220 °C.

Altering the desorption temperature, within the range 220-160 °C had no significant effect on s-triazine peak area. However below 160 °C, peak areas begin to decrease and blank desorptions at the maximum fibre operating temperature (220 °C) indicate sample carry-over for all four herbicides. This is expected because of the semi-volatile nature of the compounds studied and their relatively high boiling points when compared to conventional volatile SPME test analytes (*i.e.* BTEX). Subsequently, the fibre desorptions were performed at the maximum 100 μm fibre operating temperature of 220 °C, since this appeared to have no adverse effect on fibre performance.

Effect of Adsorption Time

The result of the investigation into the effect of the sample adsorption time on the extraction capability of the 100 μm fibre are shown numerically in table A3.9 (appendix 3) and graphically in figure 5.10 overleaf.

It can be seen from the graph that after an initial steep rise as adsorption time is increased, the peak area increases less dramatically and after 15 minutes is approaching a plateau. However, for all of the s-triazines studied, the peak area is still

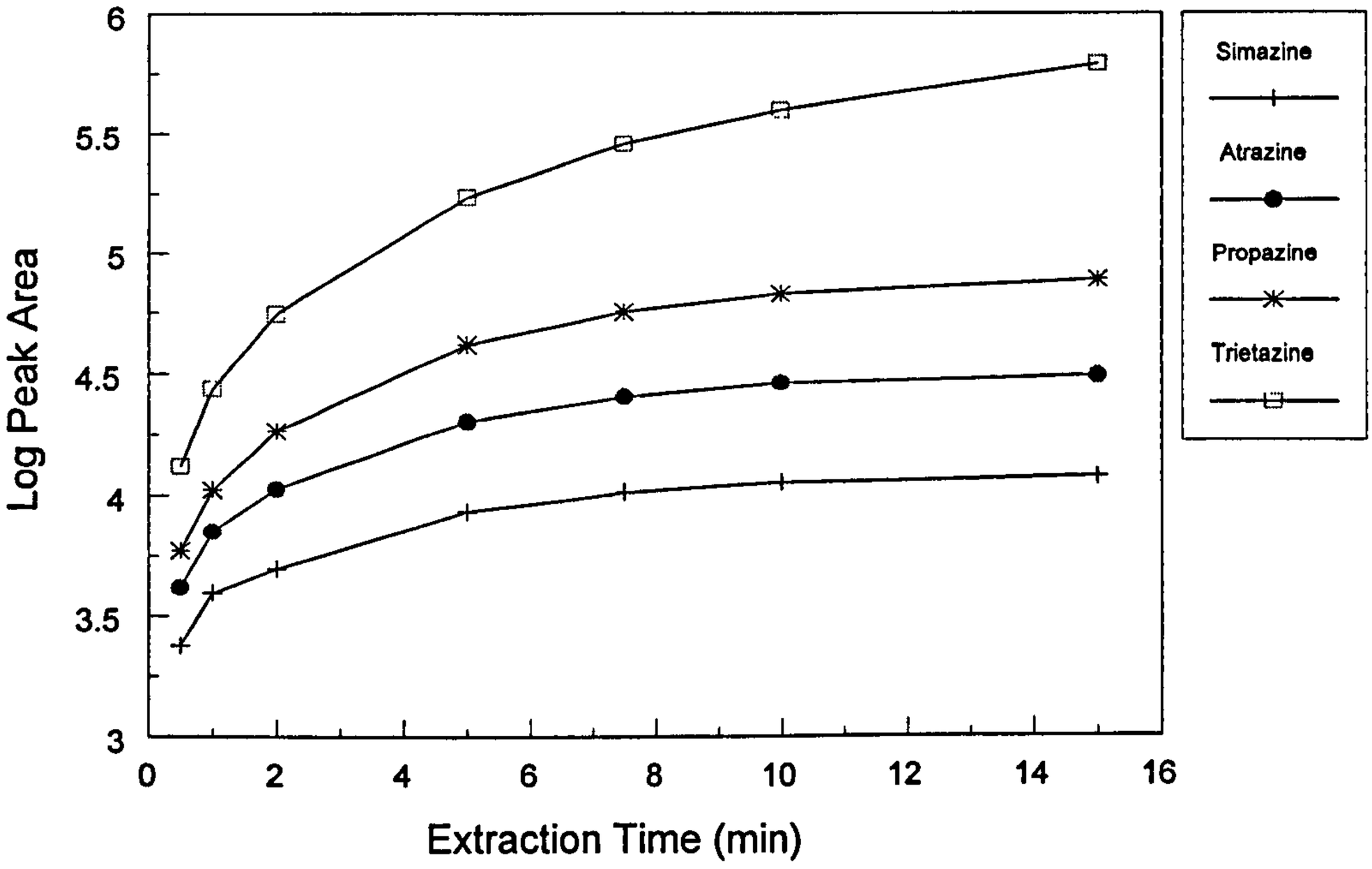


Figure 5.10 Effect of Adsorption Time on Peak Area using a 100 μm Fibre.

Conditions: 1 μg ml^{-1} solution, desorption time 15 minutes at 220 °C.

rising beyond a 15 minute adsorption indicating that the system is not yet in equilibrium. In SPME, the rate at which analytes are extracted from the matrix is determined by the efficiency with which the sample is agitated, with the exact time dependent on the agitation rate and the partition coefficient of the analytes and the fibre coating. A relatively long equilibration time is therefore expected since the sample is not perfectly agitated (where the equilibration time would be approximately 1 minute) and could be decreased if the sample was mechanically mixed. However, for practical SPME purposes, the adsorption times can be shortened to less than the equilibration time (maximum sensitivity) and are dependent on the level of sensitivity required. The adsorption time was not extended beyond 15 minutes as the peaks obtained, especially for trietazine (at the 1 μg ml^{-1} level), were so large that accurate integration became difficult.

Investigation of the Dynamic Range of the 100 μm Fibre

Three series of working calibration standards were used to investigate the linear dynamic range of the 100 μm fibre, which were run on three consecutive days. The linear correlation coefficient, for the three separate experiments, was on average 0.978. Although this value is slightly below that expected, it indicates that the fibre is linear over the concentration of 1 - 0.001 μg ml^{-1}. Unfortunately, SPME suffers from

a similar problem to that of static headspace extraction in that the technique is incapable of exhaustive extraction which in turn makes calibration difficult.[259] The poor correlation coefficient may also be partly due to the need to run the experiments on three different days where operating conditions may vary. In the absence of any agitation, the amount of analyte adsorbed onto the fibre is dependent on the diffusion of the analytes through the aqueous matrix. Therefore, this also depends on the temperature of the sample which was not maintained under isothermal conditions. Thus the fibre linearity may have improved if some form of sample agitation was used.

Below the bottom standard (0.001 μg ml^{-1}), the peaks obtained were very small and it was not possible to integrate them with any precision. Increasing the bead current of the NPD detector would have increased its sensitivity and may have allowed a lower concentration in solution to be detected but this would have been at the expense of the bead lifetime which is severely reduced when the detector is operated at elevated currents.

Multiple Extractions and Analysis of Low Concentration Solutions

In order to extract and analyze s-triazine herbicides at the EEC maximum acceptable concentration limit of 0.1 μg l^{-1} (in drinking water), multiple extractions were performed on the same sample. Initial experimentation was undertaken to reduce the required desorption time and therefore the overall multiple extraction time of analysis. In the experiment, where a 15 minute fibre desorption was compared to that of only a 5 minute desorption, no significant difference in peak areas after the two different desorptions was noted (appendix 3, table A3.10). The blank subsequently carried out after the 5 minute desorption indicated a small amount of sample carry-over (< 1 %) for propazine and trietazine with the shorter desorption time. Although this is not desirable in single extractions, it is irrelevant when performing multiple extractions since all of the analyte is trapped at the front of the column. The 5 minute desorption time at 220 °C was subsequently used in the multiple adsorption experiments.

The overall results of the multiple extraction of a 0.1 μg l^{-1} solution (repeated ten times) are shown in table A3.11 (appendix 3). The precision of the results (n = 3) ranged from 6.0 % RSD for propazine to 19.7 % RSD for atrazine. Although the RSD values are rather high, the results indicate the feasibility of the multiple extraction technique (performed automatically) to detect low concentration solutions by SPME which would normally be well below the limit of detection of the detector used.

SPME has been shown to be a simple and elegant technique which requires no organic solvent and only minor modification of existing laboratory hardware to perform fully automated extraction and analysis. SPME can not only be used to extract volatile analytes, routinely analyzed by headspace or purge and trap procedures, but is also capable of extracting semi-volatile analytes often extracted using SPE, at a fraction of the cost (the fibres used in the study have been reused approximately 100 - 150 times without deterioration in performance). An initial investigation into the principle SPME operating parameters affecting system performance was undertaken using a high concentration test sample (1 $\mu g\ ml^{-1}$) of the four s-triazines studied. This was followed by the use of a multiple adsorption technique for the extraction of the same analytes at the sub-ppb level required to analyze herbicides in drinking waters.

Chapter 6

Selective Extraction from an Aqueous Matrix

6.1 Selective Extraction from an Aqueous Matrix

There are many advantages in using supercritical fluids to extract organic analytes from environmental matrices, including an increase in the mass transfer of analytes through the fluid, leading to a more rapid extraction, and a reduction in the amount of organic solvent used. However, perhaps the most unique property of supercritical fluids is that their solvating power can be altered by changes in fluid density which is controlled by the pressure and temperature of the extracting fluid. This leads to the possibility of fractionation and class selective extraction, which may be enhanced if the fluids solvation properties are changed by the addition of a modifier or by changing to an alternative fluid with a different polarity. Despite this interesting and potentially useful property, little work has been published on the subject. Selectivity between low and high molecular weight hydrocarbons has been achieved by either increasing the density of the carbon dioxide extracting fluid[337] or by changing the extracting fluid to one with a greater solvent strength.[176] More recently, a novel additive (ionizable crown ether) was used as an extractant in supercritical carbon dioxide to selectively remove mercury from other divalent metal ions (Cd^{2+}, Co^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+}, and Zn^{2+}).[338] Selectivity using supercritical fluids as a mobile phase in chromatography has also been demonstrated.[193]

In this chapter, selectivity between organochlorine pesticides and both organophosphorus pesticides and two classes of herbicide is demonstrated. In both cases the analytes are extracted from an aqueous matrix by using solid-phase extraction disks prior to selective elution by supercritical carbon dioxide.

6.2 Selective Extraction of Organochlorine and Organophosphorus Pesticides from Water using Solid-Phase Extraction- Supercritical Fluid Extraction

In the majority of routine environmental drinking water determinations, organochlorine pesticide analysis is required together with organophosphorus pesticide analysis from the same sample. However, because of the extreme sensitive nature of the electron capture detector (routinely used for organochlorine pesticide quantitation) towards matrix interferences, sample clean-up of organochlorine samples (often including PCBs) is usually required. This is not necessary for samples requiring organophosphorus analysis only. In addition, the nitrogen-phosphorus detector, used for organophosphorus determinations, does not favour the presence of

any chlorinated compounds which may affect the bead performance. Therefore two separate samples are often used to analyze the two different classes of pesticide.

The unique properties of supercritical fluids have been used to selectively extract organochlorine pesticides from organophosphorus pesticides from the same aqueous sample after adsorption onto a solid-phase extraction disk. The class separation has been based on the analytes differences in polarity and thus solubility in supercritical carbon dioxide.

Procedure

All six of the pesticides studied (lindane, aldrin, dieldrin, dichlorvos, diazinon, and malathion, structures shown in appendix 1) were prepared in acetone and spiked into a 200 ml ± 2 ml distilled water sample at a 200 μg level. The solid-phase extraction disk procedure described in section 5.4 was then used to remove the pesticides from the aqueous solution before selective supercritical fluid elution.

The Carlo Erba fixed restrictor SFE was used for all disk extractions, together with a 10 ml extraction cell and the modified collection assembly (approximately 5 ml of hexane used as a collection solvent). As before, the flow-rate through the restrictor was kept at approximately 2 ml min^{-1} regardless of extraction pressure by altering the internal diameter of the stainless steel restrictor tubing at its tip. At the beginning of each extraction a 10 minute period of static extraction, where no actual flow of fluid occurred, was found to be essential to allow sufficient modifier-sample-analyte interaction. 30 ml of CO_2 (monitored at the pump) was then passed dynamically through the cell which took approximately 15 minutes. The extractions were undertaken at nine different pressures (using nine separate disks) in experiments ranging between 7.5 MPa to 40 MPa, with the extracts being made up to a 10 ml final volume with hexane. Finally, demeton-s-methyl was added as an internal standard. The extraction temperature was maintained at 50 °C throughout all experimentation. After extraction with pure CO_2 at the various pressures, the disks were removed from the cell and retained for further extraction. The same disks were then re-extracted in an identical manner with the addition of 400 μl of methanol, spiked directly onto the disk (whilst in the extraction cell).

Analysis of all extracts was performed using GC-MS under the conditions described in section 4.8.

Results and Discussion

The six pesticides used were chosen as a selection of common organochlorine and organophosphorus pesticides. The physical and chemical properties of the two classes are quite different and their marked difference in polarity was used to obtain selectivity in extraction. The difference in polarity is probably best illustrated by inspection of the octanol / water partition coefficient data for the six compounds which gives an indication of the hydrophobicity of the pesticides. The Log P values for the compounds (determined by the shake-flask method) are given in table A4.1 (appendix 4).[47] The non-polar nature of carbon dioxide is directly suitable for the extraction of non- and moderately-polar compounds and therefore should be capable of extracting OCPs at relatively low density (corresponding to low solvent strength of the supercritical fluid). However, because of their higher polarity, indicated by their lower Log P values, the OPPs may require the addition of a polar modifier and an increase in fluid density to enable their quantitative removal from the C_{18} SPE disks. It is these differences which can be utilized to selectively extract OCPs from OPPs.

The results obtained from the initial nine extractions involving CO_2 only are shown graphically in figure 6.1 and in full in table A4.2 (appendix 4). The distinct difference in the amount extracted between the OCPs and OPPs is immediately evident from figure 6.1. Recoveries close to 100 % are possible for two out of three OCPs at pressures as low as 13.5 MPa (dieldrin is only recovered to around 70 % at this pressure), which corresponds to a density of 0.66 g ml^{-1}. In comparison, recoveries of around 10 % or less are observed for all of the OPPs at this density. The "threshold pressure" (termed by Giddings[74]) is shown to be around 10 MPa for the OCPs where a large increase in the amount extracted is observed at this pressure. Below this pressure, non of the compounds are extracted to any significant amount. As expected, as the pressure (and consequently the density) of the carbon dioxide is increased, an increase in the overall recovery of all pesticides is observed. However although quantitative recovery of all OCPs is achieved rapidly, the maximum recovery of any OPP, even at high extraction pressure, does not exceed 80 % (malathion). After each disk was extracted with pure CO_2, a second extraction was performed on the same disks using identical extraction conditions with the addition of methanol as a fluid modifier. The recovery data of this second study are shown in appendix 4 (table A4.3), where the overall recovery from both extractions (initial extraction with CO_2 only and CO_2 + methanol modified extractions) are shown in bold type. Recoveries of OCPs from this second modified extraction are low since they have already been quantitatively removed from the disks in the first extraction with CO_2 only. The

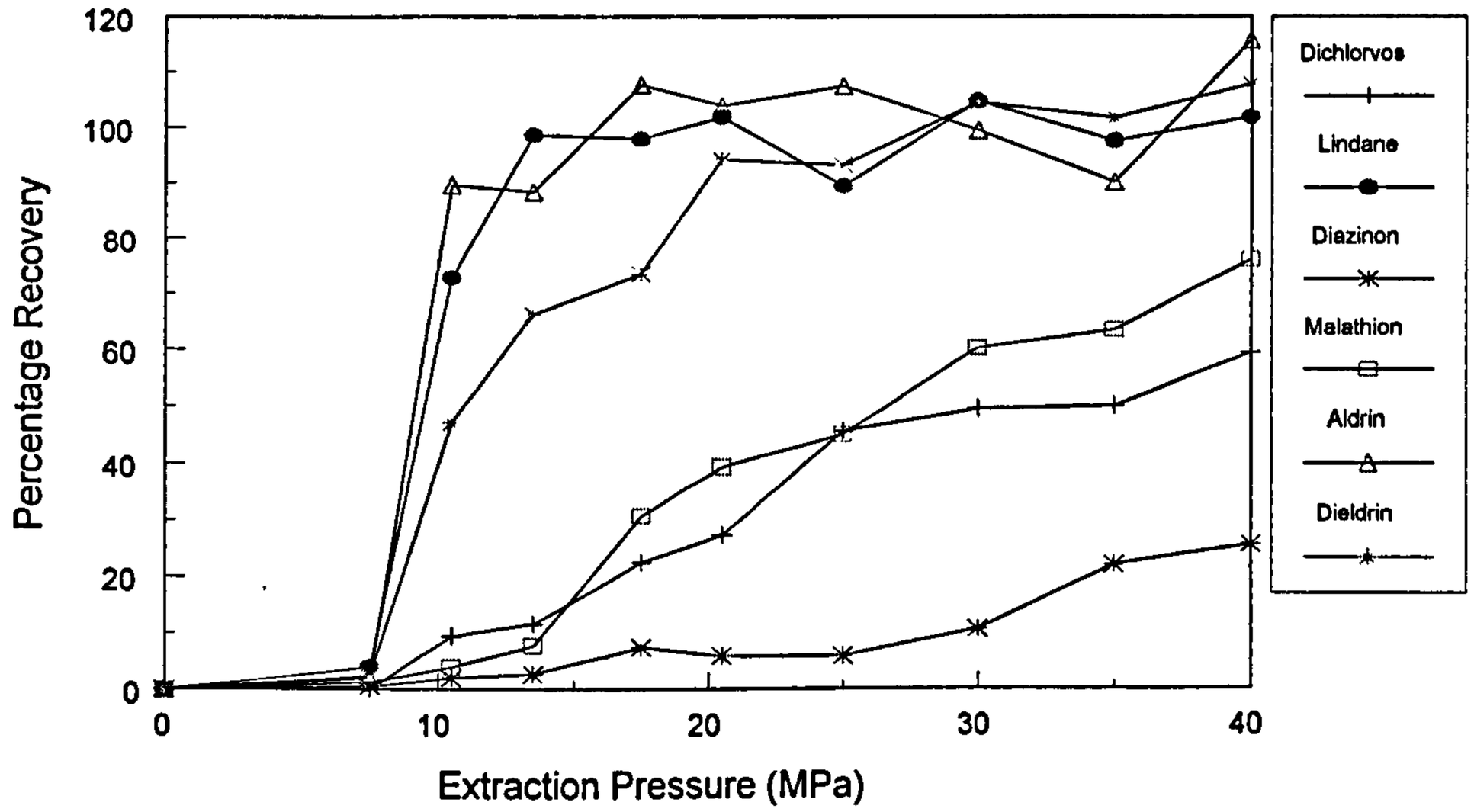


Figure 6.1 Percentage Recovery *versus* Extraction Pressure for OCPs and OPPs with CO_2 only.

cumulative recoveries are shown as a function of extraction pressure in figure 6.2 which illustrates the effect of the addition of methanol. Here it is observed that extraction efficiencies of almost 100 % can be achieved for all three OPPs with the combination of the addition of an organic modifier and the use of high extraction pressures.

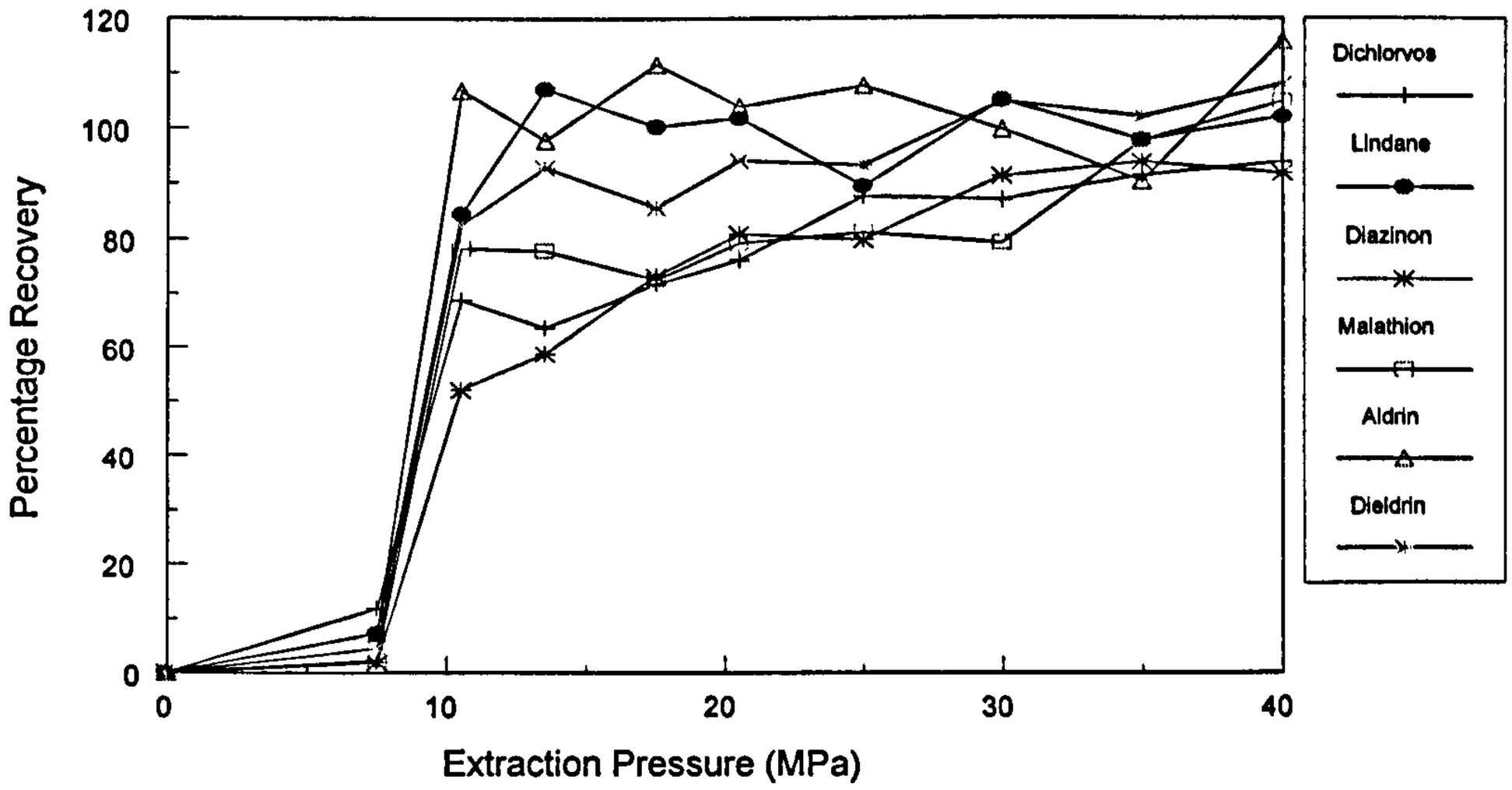


Figure 6.2 Percentage Recovery *versus* Extraction Pressure.
Cumulative Recoveries for both CO_2 only and CO_2 + Methanol Extractions.

From the analysis of the data it was deduced that the optimum conditions required to selectively extract OCPs from OPPs would be to initially extract the disk with pure CO_2 at 13.5 MPa (density 0.66 g ml^{-1}) and then to perform a subsequent extraction on the same disk with the addition of 400 µl of methanol and at a pressure of 35 MPa (density 0.93 g ml^{-1}). This extraction procedure was repeated five times to obtain an average and a standard deviation for the extraction which are shown in table 6.1.

COMPOUND	**LINDANE**	**ALDRIN**	**DIELDRIN**
Mean Recovery	91.4	98.1	72.0
Individual Recovery	86.1, 96.5, 98.2, 82.3, 94.1	103.8, 107.7, 94.7, 89.6, 94.7	68.8, 70.2, 68.2, 76.3, 76.5
Standard Deviation	6.9	7.4	4.1
% RSD	7.5	7.6	5.7
COMPOUND	**DICHLORVOS**	**DIAZINON**	**MALATHION**
Mean Recovery	95.6	93.0	84.6
Individual Recovery	92.8, 91.6, 88.7, 100.4, 92.5	89.7, 93.6, 91.2, 102.5, 87.9	82.2, 85.5, 86.1 83.3, 85.9
Standard Deviation	6.3	5.7	1.8
% RSD	6.6	6.1	2.1

Table 6.1 Recoveries of Organochlorine and Organophosphorus Pesticides after Selective SPE-SFE.

Near quantitative recoveries are obtained for all but two of the pesticides, with dieldrin only being recovered on average to 72.0 % and malathion only recovered to 84.6 % on average. In fact in figure 6.1, it is shown that dieldrin is not quantitatively recovered until an extraction pressure of approximately 25 MPa is used. In the case of dieldrin, the low recovery may be explained by the presence of the epoxide group in the molecule which allows dieldrin to behave less hydrophobically than lindane or aldrin. The reduction in the hydrophobicity is perhaps best illustrated by reference to the octanol / water partition coefficient for dieldrin in comparison with that of aldrin, (having an almost identical structure with the exception of the epoxide ring) which are 4.32 and 6.50, respectively (table A4.1). This difference in polarity can affect the solubility in pure CO_2. King illustrated this by calculating the difference in the solubility parameter for aldrin, lindane and dieldrin to be 9.5, 11.8, and 12.9, respectively.[79] In a similar manner, the solubility parameter for carbon dioxide at a pressure 340 atm and temperature of 80 °C was calculated to be 7.4 (the solubility of a compound in a supercritical fluid is maximized when its solubility parameter is similar to that of the extracting fluid). Therefore of the three OCPs, dieldrin is less likely to be extracted with CO_2 at moderate density.

One other possible explanation for the low recovery of dieldrin is in the solid-phase extraction stage where the epoxide ring may selectively hydrogen bond with any free silanol groups present on the silica particles within the PTFE membrane of the extraction disk. This could cause the complete removal of dieldrin to become difficult with pure CO_2 alone. However, with reference to table A4.3 (appendix 4), 26.8 % of dieldrin is recovered at 13.5 MPa with addition of methanol as a modifier after prior elution with CO_2 only. In this case, methanol may act in a competitive manner towards the bound dieldrin and the silanol groups allowing its release and extraction into the bulk phase.

Unfortunately, in any selective fractionation process, 100 % selectivity is seldom achieved. The affinity of malathion for pure CO_2 may explain its low recovery when selectively extracting in from a mixture of OCPs and OPPs and is demonstrated in figure 6.1 where out of the three OPPs, it is the most favourably extracted with CO_2 alone. This relatively high solubility in CO_2 means that almost 10 % of the initial concentration of malathion is extracted during the first OCP extraction stage with pure CO_2. If this is amount is combined with the 85 % (average) extracted after the second modified extraction then quantitative recovery of malathion is achieved. The slight carry-over of both OPPs and OCPs can be observed with reference to the GC-MS chromatograms in figures 6.3 and 6.4. Figure 6.3 shows the selected-ion monitoring trace for OCPs when extracted at 13.5 MPa with CO_2 only (figure 6.3a) while figure 6.3b (a baseline magnification of the previous chromatogram) shows the low extraction efficiency of the more polar OPPs obtained under these conditions. Conversely, figure 6.4a shows the selected-ion monitoring trace for OPPs when extracted at 35 MPa with CO_2 and methanol and the low residual recovery of OCPs (figure 6.4b, showing the expanded baseline of the previous chromatogram). This clearly demonstrates the ability of SFE to selectively extract OCPs with only slight extraction of OPPs at moderate density (0.66 g ml^{-1}). In contrast, the addition of methanol as a modifier allows the extraction of the more polar OPPs at higher density (0.93 g ml^{-1}) with only minimal residual extraction of OCPs which have already been removed with pure CO_2.

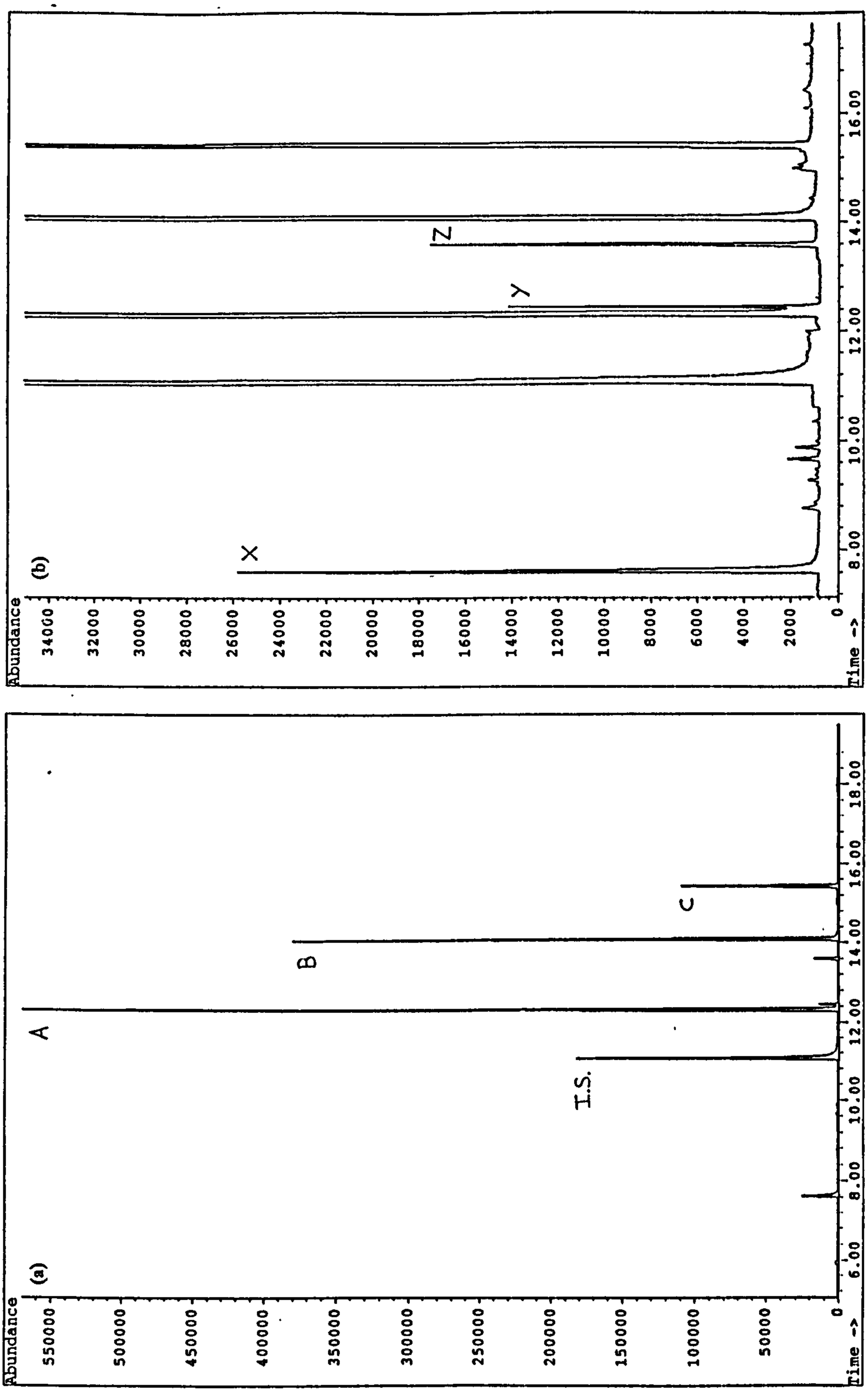


Figure 6.3 (a) Selected-Ion Monitoring of OCPs After Extraction (13.5 MPa) With CO_2 only. (IS = internal standard; A = Lindane; B = Aldrin and C = Dieldrin). (b) Selected-Ion Monitoring of OPPs After Extraction With CO_2 only. (X = Dichlorvos; Y = Diazinon and Z = Malathion).

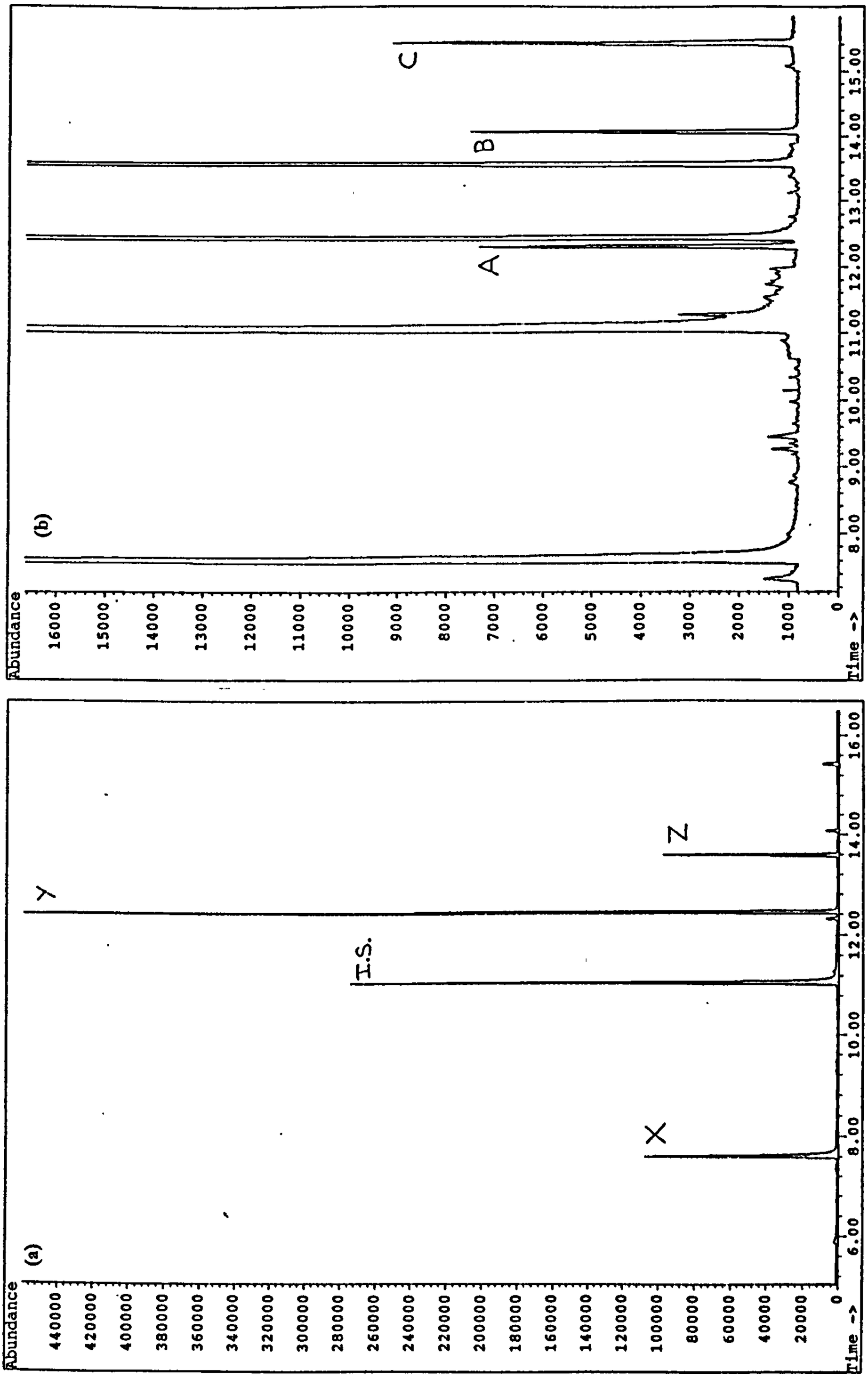


Figure 6.4 (a) Selected-Ion Monitoring of OPPs After Extraction (35 MPa) with CO_2 + 400 µl Methanol. (IS = Internal Standard; X = Dichlorvos; Y = Diazinon and Z = Malathion). (b) Selected-Ion Monitoring of OCPs After Exhaustive Extraction With CO_2 + Methanol. (A= Lindane; B = Aldrin and C = Dieldrin).

6.3 Selective Extraction of Organochlorine Pesticides and both s-Triazine and Urea Herbicides from Water using Solid-Phase Extraction-Supercritical Fluid Extraction

As with organophosphorus pesticides, different classes of herbicide often require analysis along with organochlorine pesticides. However, although some classes of herbicide are capable of being analyzed by GC without derivatization (s-triazines) others, such as the (phenyl)urea herbicides are commonly quantified by liquid chromatography because of their thermal instability which makes them unsuitable for direct GC injection. In comparison, OCPs do not give a UV response and are routinely analyzed by GC (with either mass spectrometric or electron capture detection). The two analysis systems require the sample to be introduced in different solvents (commonly hexane for GC-ECD and methanol or the actual mobile phase in HPLC) and consequently two separate samples are usually required to determine both OCPs and herbicides.

Supercritical fluid extraction has been used to selectively extract three OCPs (heptachlor, isodrin, and dieldrin) from two different classes of herbicide; s-triazines (simazine, propazine, and trietazine) and urea herbicides (chlortoluron, isoproturon, and diuron) in the same aqueous sample (all structures shown in appendix 1). The three OCPs were chosen to determine whether dieldrin is still poorly extracted (as observed in section 6.2) when compared to two different OCPs. The herbicides represent a cross-section of those routinely analyzed in drinking water samples.

Procedure

Initial extractions were concerned with the herbicides only since previous work (section 6.2) indicated that a selection of OCPs may be extracted from solid-phase extraction disks with pure CO_2 under moderate pressure conditions.

A great deal of literature is available concerning the successful extraction of s-triazine herbicides from aqueous samples using SPE technology (refer to SPE application section). However, published research on the use of solid-phase sorbents to extract urea herbicides is in comparison, relatively limited and a study was undertaken to determine the efficiency of C_{18} Empore disks (used in the conventional manner) to extract the three urea herbicides chosen.

Prior to using supercritical carbon dioxide as the elution medium from the SPE disks, a conventional organic solvent (methanol) was used elute the three urea herbicides which had been previously spiked (in methanol) into a 200 ml ± 2 ml distilled water sample at a 250 μg level. Both the sample and disk pre-conditioning was performed in an identical way to that described in section 5.4. After the sample had been passed through the disk, the disk was allowed to air dry (under vacuum) for 10 minutes. A test-tube (20 ml) was placed into the Buchner flask of the Millipore filtration apparatus and used to collect the elution solvent. The retained analytes were then eluted under vacuum with 10 ml of methanol in around 2 minutes. The extract was transferred to a 25 ml volumetric flask and made up to the mark with methanol, giving a final concentration (for 100 % recovery) of 1 μg ml^{-1}. The procedure was repeated again with the extracts being analyzed by HPLC under the conditions described in section 4.9.

The initial organic solvent elution procedure was followed by SFE experimentation, where pure CO_2 was used to extract all six herbicides from a 200 ml ± 2 ml distilled water sample (fortified at the 10 μg level). The aqueous sample was filtered through a C_{18} SPE disk by the procedure detailed in section 5.4, whereupon the dried disk was extracted with CO_2 at a pressure of 13.5 MPa using the Carlo Erba SFE. This pressure, which has been experimentally determined, is already known to successfully extract OCPs (section 6.2). A 30 minute static extraction period was used to allow adequate fluid-sample interaction and was followed by 30 ml of CO_2 passed dynamically over the sample in approximately 15 minutes. All supercritical fluid extractions (regardless of the instrument used) were undertaken at 50 °C. After completion of the first extraction, the same disk was re-extracted under identical conditions to the first extraction.

Further extractions (new samples) were performed with methanol-modified CO_2 at an increased pressure of 40 MPa, with 400 μl of methanol being added directly to the disk contained in the 10 ml extraction cell. The amount of methanol was kept at a low volume as it was found that any increase caused severe restrictor blockage during CO_2 depressurization and therefore greatly reduce dynamic flow-rates. A second extraction was performed on the same disk, under identical conditions, where a further 400 μl of methanol was added prior to commencement of extraction. All herbicide extracts were collected in the modified collection unit using the HPLC mobile phase (55 : 45 methanol : water) as the collection solvent, as any deviation from this injection solvent was found to significantly affect the resultant chromatography.

All subsequent extractions were carried out using the variable restrictor Jasco SFE system, which does not suffer from restrictor blocking due to modifier addition. Also, the Jasco has a second pump that allows modifier to be continually added to the cell during the dynamic extraction. This system was used to selectively extract OCPs (at the 100 μg level to allow GC-MSD detection) from the herbicides. The SPE procedure discussed previously was used to trap all nine OCPs and herbicides onto the C_{18} extraction disk. The disk was then extracted at 250 kg cm^{-2} with CO_2 only at a flow-rate of 2 ml min^{-1}. The extraction was carried out for a 15 minute static period followed by a 40 minute dynamic period. The extract containing the OCP fraction was collected in hexane (using the modified collection unit), transferred to a 10 ml volumetric flask, and an internal standard (β-endosulphan at a 10 μg ml^{-1} concentration) added. The disk was then re-extracted under identical conditions (with the exception of the dynamic extraction period which was reduced to 30 minutes) with the addition of 10 % methanol modifier from the second pump. The herbicide fraction was collected in the HPLC mobile phase.

Results and Discussion

The recoveries obtained from the initial experiment involving elution of urea herbicides using methanol are shown in table A4.4 in appendix 4. The extraction efficiency for all three herbicides (chlortoluron, isoproturon, and diuron) is, on average, above 100 % indicating that C_{18} Empore disks are capable of retaining the analytes under the conditions used. The high recoveries observed for isoproturon (average 126 %) are probably due to chromatographic integration problems caused by a "hump" in the baseline around the retention time of isoproturon. This was most likely due to injection of the samples in pure methanol as the hump disappeared during later analysis when samples were injected in the HPLC mobile phase.

The Empore disks were then used to extract all six herbicides from a 200 ml water sample and were eluted with pure CO_2 at an extraction pressure known to quantitatively extract OCPs (13.5 MPa). The results of the study are shown in table A4.5 (appendix 4). Even after two long extractions (a 30 minute static period followed by 30 ml of CO_2 in dynamic extraction), a maximum of only 5 % is extracted for any of the herbicides. It is obvious from these results that CO_2 alone is ineffective in removing the herbicides from the extraction disk. This is primarily due to the low solubility of the relatively polar herbicides in non-polar CO_2. Once again this can be used to selectively remove OCPs from the more polar herbicide fraction. The

differences in polarity between the OCPs and herbicides can be illustrated by the octanol / water partition coefficients (Log P) of heptachlor and simazine, which are approximately 5.5 and 2.0, respectively.[47] The smaller Log P value of simazine indicates that it is relatively polar, are therefore its extraction with CO_2 will require a modifier to increase the solvating power of the fluid. However, as before, the OCPs are non-polar (higher values of Log P) and should be removed effectively with CO_2 only.

In an attempt to quantitatively extract the herbicides, a methanol modifier (400 µl) was then added directly to the disk in the extraction cell, and the extraction pressure was increased to 40 MPa corresponding to a density of 0.928 g ml^{-1} (the extraction was carried out for an equal length of time). The procedure was then repeated, on the same disk, with the addition of a second aliquot of methanol. The recoveries of the two sequential extractions obtained using methanol-modified CO_2 are shown in table A4.6 (appendix 4). The table shows that recoveries for the first extraction lie between approximately 65 % and 85 % depending on the analyte. After a second extraction, where more methanol is added, the total recoveries are around 90 %. However, it was not possible to achieve this near quantitative recovery in the first extraction because as soon as the dynamic extraction period was started, the methanol modifier was flushed from the cell and the herbicides were no longer effectively extracted with the pure CO_2. The addition of a greater volume of methanol was restricted because increasing the methanol content caused the Carlo Erba's fixed restrictor to become blocked. Also an extended static extraction period, in which the actual modifier-sample-analyte interactions occur, was impractical because an already lengthy static extraction was being used (30 minutes) and any addition would have caused the overall extraction to become overly long. The Jasco SFE was therefore used for all subsequent extractions because it incorporated not only a variable restrictor which did not suffer from blockage problems, but also a second pump which enabled modifier to be continually delivered throughout the dynamic extraction. This allows contact between the modifier and the sample in both the static and dynamic periods of the extraction.

The Jasco SFE was then used to elute all nine analytes (three OCPs and six herbicides) from the C_{18} SPE disks. An initial extraction was performed with CO_2 only at a pressure of 250 kg cm^{-2}, a temperature of 50 °C (a density of 0.85 g ml^{-1}), and a flow-rate of 2 ml min^{-1}. The higher density, compared to that of 0.66 g ml^{-1} for the OCP extraction reported in section 6.2, was used because almost no herbicides were found to be extracted with CO_2 only (table A4.5) and therefore the higher density has no real effect on the overall degree of selectivity obtained. In addition, the

increase in the fluid solvent strength was used to enhance the ability of CO_2 to extract dieldrin which was not quantitatively extracted at 13.5 MPa in the work detailed previously (section 6.2). The whole procedure was repeated five times in ascertain the precision of the extraction. The results of the first extraction are summarized in table 6.2, with the full results shown in appendix 4 (table A4.7).

A typical chromatogram obtained from the analysis of an OCP standard (10 μg ml^{-1}) by GC-MSD is shown overleaf in figure 6.5.

Compound	Mean Recovery (%)	Percentage RSD ($n = 5$)
Heptachlor	91.7	2.3
Isodrin	101.6	8.1
Dieldrin	84.8	2.8
Simazine	1.6	76.8
Propazine	2.7	168.0
Trietazine	3.5	98.4
Chlortoluron	4.4	69.6
Isoproturon	3.9	54.0
Diuron	4.3	61.7

Table 6.2 Summary Table of Percentage Recoveries of OCPs and Herbicides using CO_2 Only. (Jasco SFE)
Conditions: 250 kg cm^{-2}; 50 °C, flow-rate 2 ml min^{-1}.

It is apparent from the results that almost none of the herbicides are recovered at this pressure with CO_2 only (the high RSD values are due to the very small percentage extracted), whereas two of the three OCPs (heptachlor and isodrin) are extracted with over 90 % efficiency. However, despite the increase in the density of the extracting fluid (when compared to that used in section 6.2), dieldrin is less than quantitatively recovered, with an average of around 85 %. However, this is higher than the amount extracted under similar conditions during the OCP-OPP selectivity study detailed in the previous section (average of 72.0 %). The increase is most likely explained by the increase in fluid density. This complements the view that it is the epoxide group in the dieldrin molecule which causes it to be inefficiently recovered since neither of the other two analytes, used in this study, have this functional group in their structure. However, the exact reason for the poor extraction capability is not known.

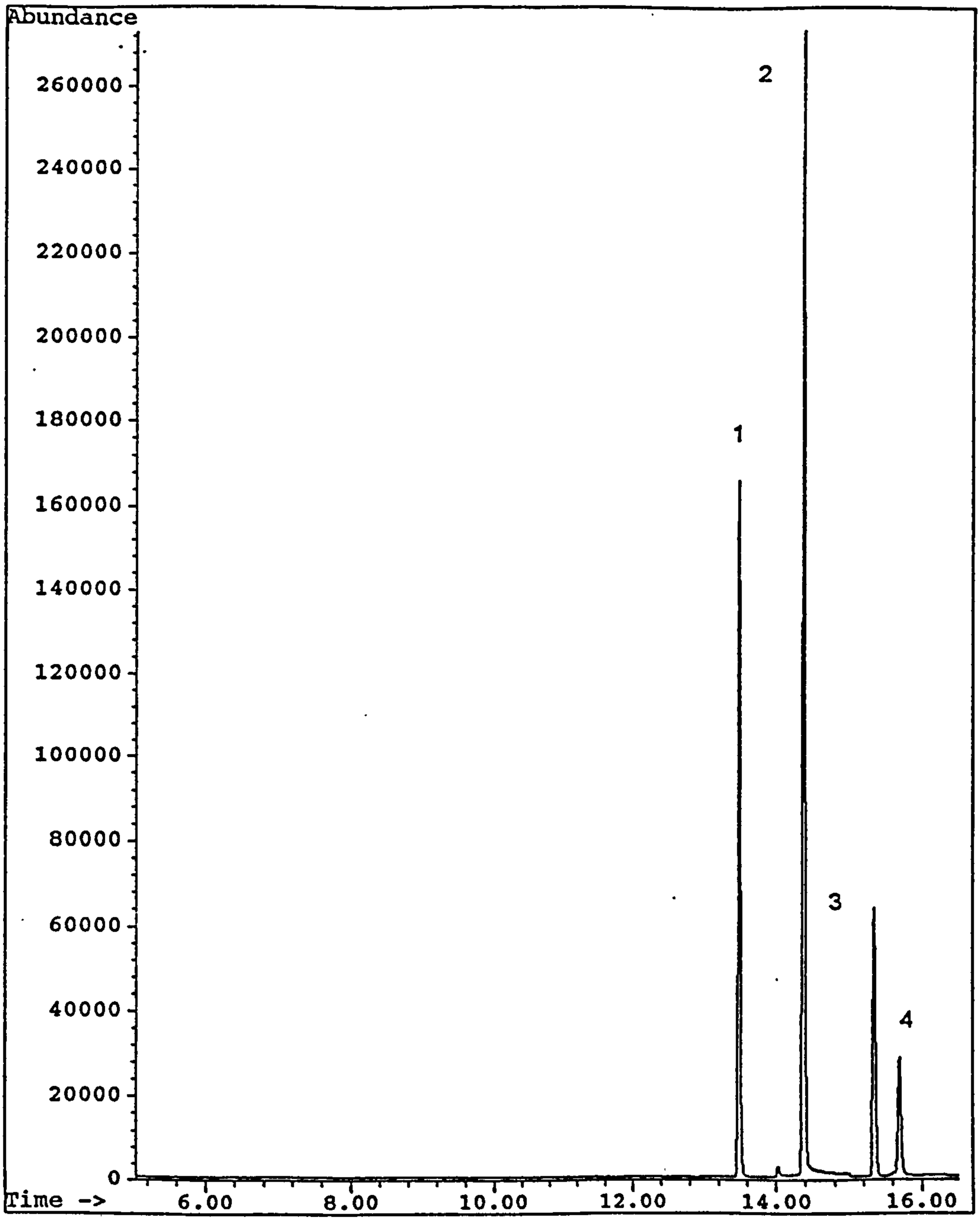


Figure 6.5 A Typical GC-MSD Chromatogram for the OCPs used in the OCP / Herbicide Selectivity Study.

(1 = Heptachlor, 2 = Isodrin, 3 = Dieldrin, 4 = Internal Standard).

All five disks were then re-extracted under the same pressure and temperature conditions with the addition of 10 % methanol as the modifier, using the Jasco SFE's second pump. The recoveries for the herbicides are shown in full in table A4.8 (appendix 4) and summarized in table 6.3.

Compound	Mean Recovery (%)	Percentage RSD ($n = 5$)
Simazine	100.4	12.9
Propazine	88.5	7.4
Trietazine	86.6	9.0
Chlortoluron	86.1	9.3
Isoproturon	90.2	5.2
Diuron	87.8	7.7

Table 6.3 Summary Table of Percentage Recoveries of Herbicides using Methanol Modified CO_2 (Jasco SFE).

Conditions: 250 kg cm^{-2}; 50 °C, flow-rate 2 ml min^{-1} and 10 % methanol modifier.

The herbicide recoveries from the second extraction are now all approximately 90 % with the continual addition of the modifier during the dynamic extraction period. This recovery does not include the small amount of herbicide removed in the first (CO_2 only) extraction. Recoveries of OCPs are not shown in the table because they were already selectively removed from the disk in the first extraction and were therefore not detected.

The results shown in tables 6.2 and 6.3 indicate that selective extraction is possible between OCPs and herbicides using SFE. Overall, better separation between the two analyte classes is obtained when compared to the selectivity between OCPs and OPPs detailed in section 6.2 with, on average, half as much of the herbicides being removed before the second extraction as the OPPs. The improved selectivity may be best illustrated by consideration of example chromatograms obtained during the herbicide extractions. Figure 6.6a shows the HPLC chromatogram for the herbicide extraction with CO_2 only, and figure 6.6b shows the modified-CO_2 extraction trace from the same disk. The difference in the HPLC chromatograms clearly indicates both the extraction selectivity of SFE and the separation / detection system.

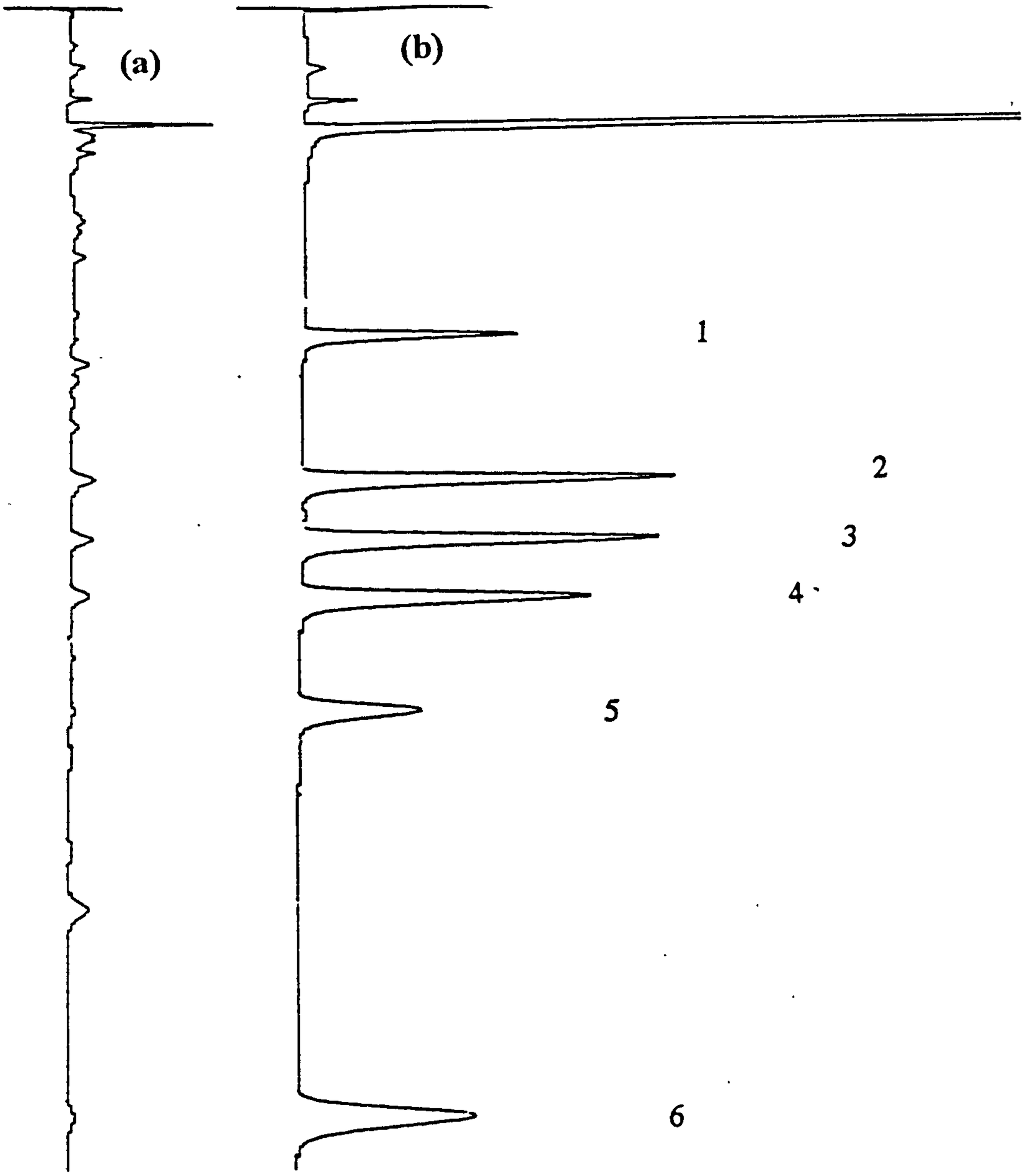


Figure 6.6 HPLC Chromatograms of Herbicide Extraction: (a), with CO_2 only; (b), with CO_2 plus 10 % methanol as a modifier.
Elution Order: (1) simazine; (2) chlortoluron; (3) isoproturon; (4) diuron; (5) propazine; (6) trietazine.

Overall, the possibility of selectively extracting OCPs from both OPPs and two different classes of herbicide has been demonstrated using a SPE-SFE approach. Whilst compromise SFE conditions were required to achieve the selectivity reported between OCPs and OPPs, the fractionation between OCPs and herbicides is almost complete. Both examples of selective extraction are obtained because of differences in the polarity between the classes of pollutant, with the non-polar compounds (OCPs) being efficiently removed using CO_2 alone. Conversely, the more polar analytes (OPPs and herbicides) require the addition of a polar modifier (methanol) to the supercritical fluid to achieve quantitative extraction. In the case of OCP-OPP selectivity, direct addition of a small quantity of the modifier to the SPE disk was found to be sufficient to remove the OPPs after the OCPs had been previously eluted with CO_2 only. However, the herbicides were shown to be more difficult to remove from the extraction disk and required the use of a SFE system incorporating a second pump to continually add modifier throughout the extraction. Selectivity of extraction may prove useful in both examples since their subsequent detection is conventionally achieved using different chromatographic systems.

Chapter 7

Extraction of Polycyclic Aromatic Hydrocarbons from Contaminated Land: A Comparison

7.1 Extraction of Polyaromatic Hydrocarbons from Contaminated Land: A Comparison

The second half of the research is devoted to the extraction of pollutants from solid samples. This is a role in which SFE has been implemented far more successfully than for removing analytes from aqueous matrices, discussed in the first part of the work. In particular, soil is used as the matrix since it is soil which, behind water, is probably the sample type requiring analysis most frequently in an environmental analytical laboratory. As well as SFE, other sample preparative techniques will be assessed which represent both traditional (Soxhlet) and more modern (microwave assisted extraction) procedures.

The extraction of polycyclic aromatic hydrocarbons (PAHs) from contaminated soil sites is of major environmental concern. Traditionally, the extraction of PAHs from soils is carried out by Soxhlet extraction and indeed, the suitability of Soxhlet extraction has never been in doubt since its inception in the 19th Century. This chapter discusses the ability of Soxhlet extraction to recover a range of PAHs from contaminated soil and is compared with newer methods of extraction (SFE and microwave assisted extraction) which may offer many advantages over this more traditional method of solvent extraction.

7.2 Polycyclic Aromatic Hydrocarbons in Contaminated Land

Polycyclic aromatic hydrocarbons are frequently found at high levels (hundreds of parts per million) in contaminated land sites and primarily arise from previous industrial processes involving fossil fuels. Examples of possible causes of contamination include old filling station sites and gas works where high levels of fuels containing PAHs (and other pollutants) have escaped into the soil phase over a period of many years. Often, the land is to be used for subsequent building and many soil samples must be analyzed to assess the extent of contamination before workers can safely operate in the area.

As in the majority of organic analysis, the rate determining step which controls the speed at which analysis can occur is the lengthy solvent extraction procedure. Typically Soxhlet extractions take several hours to complete and use large volumes of organic solvents, which themselves cause environmental disposal problems. Soxhlet extraction has been so widely used that it is seen as producing an extract which

contains an "accurate" representation of the true concentration. It is therefore used as a bench-mark when assessing other sample preparative methods being used to extract analytes from solid samples. Two different sample preparation techniques (microwave assisted extraction and SFE) have been compared to Soxhlet extraction for the removal of sixteen PAHs, as defined according to the US Environmental Protection Agency (structures shown in appendix 1). Experimental design is used in both cases to optimize the various operating parameters thought to affect the extraction efficiency.

"Real" contaminated land soil samples are preferred over laboratory prepared samples throughout the investigation as they give a true representation of the efficiency of an extraction technique to remove analytes from strongly binding sorbent sites. In addition, contaminated land soils are often regarded as one of the most difficult matrices to extract as they can contain a large variety of pollutants as well as the analytes of interest. The matrix itself can also vary greatly in its composition and contain appreciable amounts of foreign material (*e.g.* metallic objects and coal) depending on its origin. Two different contaminated land soils were used in the study at low and high PAH concentration. A third soil, used in a Laboratory of the Government Chemist (LGC) inter-laboratory evaluation (CONTEST), was also employed.

7.3 Soxhlet Extraction

The principles behind Soxhlet extraction have been discussed in section 2.2.2.1 and are widely accepted as the standard technique used for extracting analytes from solid samples, so much so that it is often used to assess the performance of other extraction methods. Extraction solvents used in the apparatus are dependent on the target analytes of interest but for PAHs are typically, dichloromethane (DCM), toluene, or cyclohexane. DCM was used exclusively in the study since it is the chosen solvent for the Soxhlet method under evaluation. In addition, neither toluene or cyclohexane may be used alone as a solvent in microwave assisted extraction (not heated by microwave energy). The Soxhlet extraction procedure detailed below, was used in repeated extractions of all three contaminated soils.

Procedure

Sample Collection and Preparation

The following procedure was used to collect and prepare the soils for all of the extraction studies:

The contaminated land soils were sampled from known sites and brought to the laboratory in air-tight wide-mouthed glass jars which had been cleaned with detergent (Decon 90), thoroughly rinsed with deionized water, acetone and dried at 105 °C. The jars were then rinsed with hexane and dried before use. After sampling, the soils were air-dried on trays at a temperature not exceeding 30 °C. All large stones and extraneous material (contaminated land samples can contain large amounts of coal) were removed by hand and the soils placed in a commercial blender where they were blended for a minimum of 5 minutes. The fine powdered soils were then stored in an air-tight container until required. The LGC test soil was used as received in a similar powdered form as the other soils.

Soxhlet Extraction

Soxhlet extractions were performed using 10 g ± 0.1 g portions of soil which were added to 30 g ± 0.1 g of anhydrous sodium sulphate and well mixed in a beaker. The sodium sulphate was used as a drying agent to remove any residual moisture from the soil and therefore facilitate the contact between the sample and the water immiscible solvent (DCM), and in addition, to disperse the soil particles and create a greater contact area between sample and solvent. The mixture was transferred into a cellulose extraction thimble containing approximately a 1 cm depth of sodium sulphate. All sodium sulphate used was previously dried in a muffle furnace at 500 °C for a minimum of four hours and stored in a dessicator prior to use. The thimble was covered with a loose wad of cotton wool (both pre-extracted with DCM) and inserted into a Soxhlet assembly, which was fitted with a 250 ml round-bottomed flask (containing 100 ml ± 2 ml of DCM) and a reflux condenser. The solvent was heated for 6 hours on an isomantle which was adjusted to allow 6-8 rinse cycles to be performed each hour. Upon cooling, the extract was transferred into a 100 ml calibrated flask where it was made up to the mark with DCM. Repeat extractions were performed simultaneously using a bank of isomantles containing six separate units.

250 µl of the extract was removed from the flask and placed, together with and equal amount of an internal standard solution (20 µg ml^{-1}), in a GC-MS autosampler vial to await analysis.

Results and Discussion

The extracted concentrations of PAHs obtained from soil 1 during a repeatability study ($n = 6$) are shown in the summary table 7.1, and in full in table A5.1 (appendix 5). All concentrations (throughout the PAH study) are shown in mg kg^{-1} of dry soil and are calculated by knowledge of the exact weight of sample extracted. Although the overall concentration of PAHs appear to be high, the individual concentrations are relatively low which accounts for the high relative standard deviation of the individual PAHs. The detection limit of the GC-MS used to analyze PAHs was 0.1 μg ml^{-1}, with the top calibration standard at 50 μg ml^{-1}, to allow for high concentrations of PAHs often encountered in contaminated land samples. Therefore the individual amounts extracted from soil 1 actually fall at the very start of the calibration plot used to calibrate the GC-MS. In addition, all analysis of soil 1, regardless of the sample preparation technique, was performed using a Finnigan Incos GC-MS (detailed in section 4.8) whose data handling software requires that peaks be manually integrated. This caused the calculated concentrations to become operator-dependent, particularly at this low concentration where baseline noise becomes evident, which can cause poor reproducibility.

A second contaminated land samples was then used throughout the majority of the comparison study, which was known to have far greater concentrations of individual PAHs, more representative of contaminated land sites. Soxhlet extractions (using identical conditions) were repeated five times with the results of the study also summarized in table 7.1 (in full in table A5.2, appendix 5).

In is apparent from the table that the overall amount of PAHs present in soil 2 has increased and is more representative of contaminated land sites commonly encountered. This is particularly true for phenanthrene and fluoranthrene who both have average concentrations of PAHs above 50 mg kg^{-1}. Consequently the RSDs obtained are substantially lower than those for soil 1, with the exception of dibenz (a,h) anthracene. Also, the use of a fully-automated GC-MS system (Hewlett Packard), for the analysis of both soil 2 and the LGC test soil, removes operator bias when carrying out peak integration. This is illustrated by a RSD value of only 1.6 % ($n = 5$) for the total amount of PAHs detected which was obtained during a GC-MS repeatability study using the Hewlett Packard instrument.

A similar study was undertaken using the LGC CONTEST soil, although was only extracted three times because of the lack of sample. During the cooling period, after

the six hour extraction was completed, yellow crystals were formed on the glass surface of the round-bottomed flask which were thought to be sulphur present in the soil (this is common in contaminated land samples). The results of the study are shown in table 7.1 (shown in full in appendix 5, table A5.3). The LGC soil (3) was found to contain slightly lower amounts of PAHs compared to soil 2, however, is still representative of levels found at contaminated land sites. Percentage RSDs are excellent for the majority of individual PAHs despite only three repeat extractions. The high RSD obtained for anthracene is once again due to the low concentration of the extracted analyte, whereas the relatively high RSD for benzo(k)fluoranthene is most likely due to integration problems since benzo(b) and benzo(k)fluoranthene co-elute under the GC-MS temperature programme used (see figure 7.1). The overall total amount of PAHs extracted by Soxhlet show excellent agreement with a relative standard deviation of 1.0 %.

	SOIL 1			SOIL 2			SOIL 3	
Compound	**Average**	**% RSD**		**Average**	**% RSD**		**Average**	**% RSD**
Naphthalene	6.0	17.5		4.2	22.4		12.1	4.7
Acenaphthylene	0.7	73.2		2.6	19.5		1.8	5.6
Acenaphthene	1.9	30.1		6.4	20.1		0.9	6.2
Fluorene	2.5	24.3		8.6	15.9		0.8	0.0
Phenanthrene	1.0	45.8		53.4	11.2		67.9	3.5
Anthracene	2.3	34.6		13.6	8.2		1.9	36.5
Fluoranthene	1.1	17.0		54.1	6.1		56.8	5.0
Pyrene	1.0	31.5		43.0	7.1		34.3	1.4
Benz(a)anthracene	2.9	35.8		25.3	7.6		10.9	0.5
Chrysene	3.9	33.4		26.6	4.9		15.5	3.8
Benzo(b)fluoranthene	2.8	26.3		15.1	19.2		13.4	3.8
Benzo(k)fluoranthene	3.4	44.1		11.0	12.0		9.6	22.8
Benzo(a)pyrene	4.1	59.5		15.3	16.6		2.1	4.8
Indeno(1,2,3-cd)pyrene	6.0	73.3		7.2	27.4		2.8	5.4
Dibenz(a,h)anthracene	12.9	90.0		3.4	58.3		1.8	3.1
Benzo(ghi)perylene	5.6	63.4		7.6	23.0		10.0	3.5
Total	58.1	43.1		297.4	10.0		242.7	1.0

Table 7.1 Results Summary of Soxhlet Extractions using Dichloromethane for all Three Test Soils.
(Concentration in mg kg^{-1})

A typical GC-MS selected ion monitored chromatogram (Hewlett Packard) for the 16 PAHs (at the 10 μg ml^{-1} level) and two internal standards (20 μg ml^{-1}) is shown in figure 7.1 overleaf.

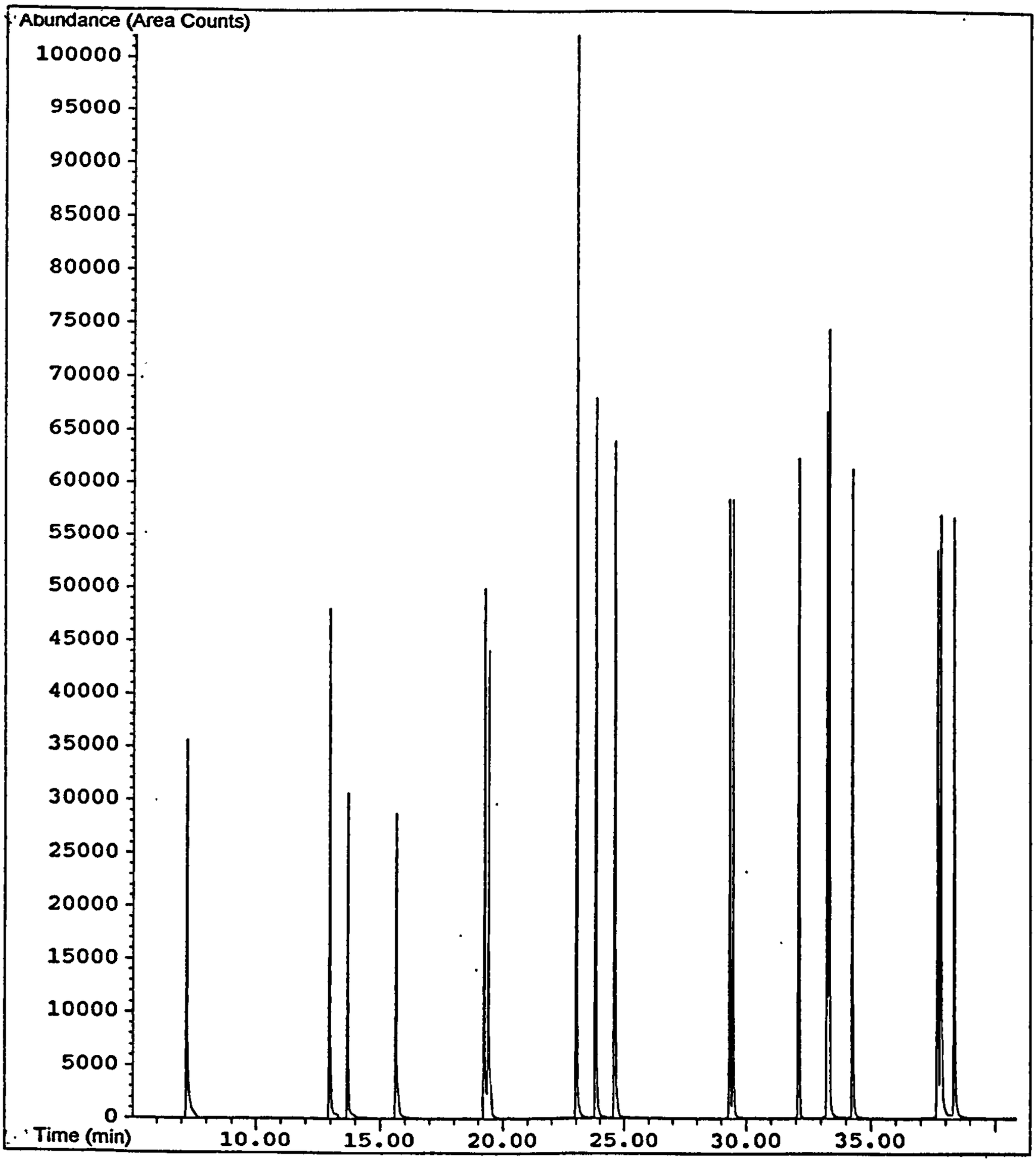


Figure 7.1 A Typical GC-MS Chromatogram of PAHs (Hewlett Packard).

Note:- The elution order of the PAHs is the same as the order in which they appear in all of the results tables in chapter 7.

(Peaks at 23.0 and 32.1 minutes are the internal standards, 3,6-dimethylphenanthrene and 6-ethyl chrysene, respectively).

The concentration of the PAHs recovered from all three soils using Soxhlet extraction can be used as a "bench-mark" level to evaluate microwave assisted extraction and supercritical fluid extraction, both of which have been used extensively to extract PAHs from soil matrices (see appropriate applications sections).

7.4 Microwave Assisted Extraction

Microwave assisted extraction has many advantages over traditional Soxhlet extraction, used in section 7.3, for the removal of analytes from solid samples. The rapid heating of solvents (in a closed vessel) at a temperature above their normal atmospheric boiling point allows the extraction of samples in minutes as opposed to hours. In addition, the commercial instrumentation utilized in the study is capable of extracting twelve samples simultaneously and allows solvent usage to be greatly reduced. It also has in-built safety features which are required when working with organic solvents. The microwave instrument used in the comparison study is described in greater detail in section 4.5.

A procedure for extracting PAHs from the contaminated soil samples (used in the previous section) using microwave energy is described. Solvent type, composition, and volume, sample size, extraction temperature and pressure, as well as the time of extraction are all studied to determine the optimum operating conditions to remove the sixteen PAHs studied.

Procedure

All microwave extractions were performed using a 1000 W microwave unit specifically designed for use with organic solvents. In the initial experiments a portion of soil (5 g ± 0.1 g) was placed into the PTFE sample vessel liner and 30 ml ± 1 ml of DCM added. However, it was found that the microwave heating of certain soil types caused electrical arcing inside the sample vessels, similar to that observed when metallic objects are placed in a microwave field. This may be due to the sample not being fully submerged in the solvent at the start of the extraction or to small particles of soil attracted by static electricity to the PTFE liner of the extraction vessel. Components in the contaminated land samples (metallic objects or coal) which can reflect microwaves may have caused the characteristic arcing inside the microwave vessels. The effect was amplified by using DCM as the extracting solvent since it is

not a particularly polar solvent and therefore is relatively inefficient at absorbing microwave energy. This leaves excess microwave energy capable of being reflected by the elements exposed on the soil surface (the problem did not occur when acetone, or acetone - hexane mixtures were used as extracting solvents). The problem was solved (for DCM) by reducing the amount of soil extracted to 2 g portions and increasing the solvent volume to 40 ml. In addition, care was taken to ensure all soil particulate matter statically attracted to the liner walls was washed with solvent into the bulk soil solution, prior to commencing extraction. This prevented any arcing inside the sample vessel by allowing all of the soil to be submerged in the solvent. The microwaves were then unable to come into direct contact with the reflective material present in some soil samples. A smaller amount of soil, together with the increased solvent volume was then used throughout the subsequent experimentation.

A 2 g ± 0.1 g portion of soil was weighed and transferred into the PTFE liner of the sample vessel where the extraction solvent (40 ml ± 1 ml) was added. Both DCM and acetone - hexane mixtures (at various compositions) were used to extract the soils. Care was taken to fit new rupture membranes in each vessel before the beginning of each extraction. The vessels were then placed symmetrically on the microwave turntable, together with a control vessel containing the pressure and temperature sensory equipment. Each vessel was connected to the central well by PTFE tubing and the extraction temperature, pressure, time, and microwave power set. Initial studies focused on the extraction of a contaminated land sample which was subsequently found to have a low concentration of individual PAHs (soil 1). Microwave extractions were carried out using DCM (40 ml) at a constant extraction temperature of 120 °C. The magnetron power was set at 50 % for an extraction time of 20 minutes (although the optimum power setting required to adequately heat a sample to its set extraction temperature in a reasonable length of time is dependent upon the nature of the solvent and the number of sample vessels being simultaneously extracted).

As in section 7.3, it was found that the individual levels of PAHs present in soil 1 were too low to be reproducibly extracted and detected. Therefore a soil containing higher concentrations, more representative of contaminated land samples, was used (soil 2). DCM microwave extractions were carried out on soil 2 together with a number of experiments involving different compositions of an acetone - hexane mixture. All of these extractions were undertaken at the same conditions as used for soil 1 with the exception of the magnetron power, which was reduced to 30 % for the acetone - hexane study, due to the ability of acetone to absorb a greater amount of microwave energy. After the extractions were completed, the vessels were allowed to

cool until no residual pressure was observed. This prevented the loss of the more volatile analytes when the sample vessels are opened. The soil / solvent mixture from each vessel was then filtered through a GF/A glass microbore filter into a 50 ml calibrated flask and the remaining solid washed with fresh solvent, which was also added to the flask prior to making up to volume.

All microwave extracted samples were analyzed by GC-MS by taking a 500 µl aliquot of extract, together with 250 µl of internal standard (20 µg ml^{-1}) and placing them in a GC-MS autosampler vial. Twice the amount of sample was used, when compared to Soxhlet extractions, since only 2 g of sample was initially extracted.

For chemical systems with multiple variables, the "single-factor-at-a-time" approach to parameter optimization often leads to an incomplete understanding of the behaviour of the system. This technique also requires many experiments to be performed which is time-consuming. Therefore experimental design, where all of the factors are varied simultaneously, was used to determine which operating parameters, if any, had a significant effect on the amount of PAHs recovered from soil 2.

Once the optimum extraction solvent had been established, a central composite design (detailed in section 3.1.4) was used to evaluate the effect of extraction temperature, time and solvent volume on PAH recovery. The three variables were input in specifically designed computer software (Design Expert, discussed in section 4.11) to elucidate the central composite design (CCD). The maximum and minimum levels of each variable were set by operating constraints and are shown in table 7.2.

	Variable 1 Temperature (°C)	**Variable 2 Extraction Time (min)**	**Variable 3 Solvent Volume (ml)**
Upper Limit	120	20	50
Lower Limit	40	5	30

Table 7.2 The Upper and Lower Limits for the Variables used in the Microwave Assisted Extraction Central Composite Design.

The complete design consisted of 20 experiments including six central repeats (to allow the pure error in the system to be determined), although the software allowed the experiments to be blocked and carried out on separate days. All extractions were

carried out as duplicates which were performed simultaneously. The full design, with the experiments shown in their run order, is listed in table A5.4 (appendix 5).

The optimum operating parameters obtained from the CCD were then used to extract the LGC soil (3) using both DCM and acetone as extracting solvents in an attempt to study the effect of a different soil type on microwave extraction.

Results and Discussion

In the initial experimentation, the low PAH concentration soil (1) was extracted using a DCM microwave extraction at 120 °C for 20 minutes. The four microwave repeat extractions were performed simultaneously and the results obtained are shown in table A5.5 in appendix 5. The results show that although a 20 minute microwave extraction is comparable in terms of PAH recovery with a 6 hour Soxhlet extraction, the precision of the replicates is poor (although appreciably better than the overall RSD obtained using Soxhlet), for reasons similar to those discussed in section 7.3. However, these initial results do show that by using microwave assisted extraction at increased solvent temperatures, it may be possible to significantly reduce the extraction time.

A second soil was used (soil 2), which contained a higher concentration of PAHs. The soil was subjected to repeat microwave extractions using identical conditions as used on soil 1. The results of the study are shown in appendix 5 (table A5.6). Once again, similar amounts of PAHs are extracted using microwave energy when compared to Soxhlet, although the precision for the microwave extracted samples is slightly poorer than that obtained during the Soxhlet repeatability study (this may simply be due to a reduction in the number of replicates performed). However, the benefits of microwave extraction are that it allows an approximate 20-fold reduction in extraction time and uses over 50 % less DCM when compared to Soxhlet.

In recent years chlorinated solvents have come under close environmental scrutiny and for this reason it was decided to investigate other, non-chlorinated solvents as possible extractants for use in microwave extraction. The most popular solvent to be used in the literature for the extraction of PAHs using microwaves is a mixture of acetone and hexane, since hexane alone is incapable of absorbing microwave energy.[304,339] For simplicity, the normal composition is a 1 : 1 ratio of the two solvents but there has been little published research into the advantages of using this

composition. A range of compositions between 80 : 20 / hexane : acetone to 100 % acetone were chosen to observe the effect of the solvent ratio on PAH recovery. As before, 2 g of the soil was extracted in duplicate with a total solvent volume of 40 ml being maintained. The temperature and extraction time remained constant (120 °C and 20 minutes, respectively), although the magnetron power was reduced to 30 % since acetone has a greater dielectric constant than DCM and is therefore heated more effectively by microwave energy. If a higher power was used it caused the solvent to reach its boiling point too quickly, resulting in the set temperature being grossly exceeded.

The results shown in table A5.7 (appendix 5) indicate that low volumes of acetone in an acetone : hexane mixture are equally as capable as DCM at extracting PAHs from soil 2. As the composition of the acetone in the mixture is increased, the total concentration of PAHs extracted is also increased and it appears that the most efficient solvent mixture for extraction of PAHs from the soil is actually 100 % acetone. This trend is illustrated in figure 7.2, which also shows the average total amount of PAHs extracted using DCM as a comparison. The figure shows that for this particular soil, acetone is capable of removing, on average, over 170 mg kg^{-1} more total PAHs than DCM.

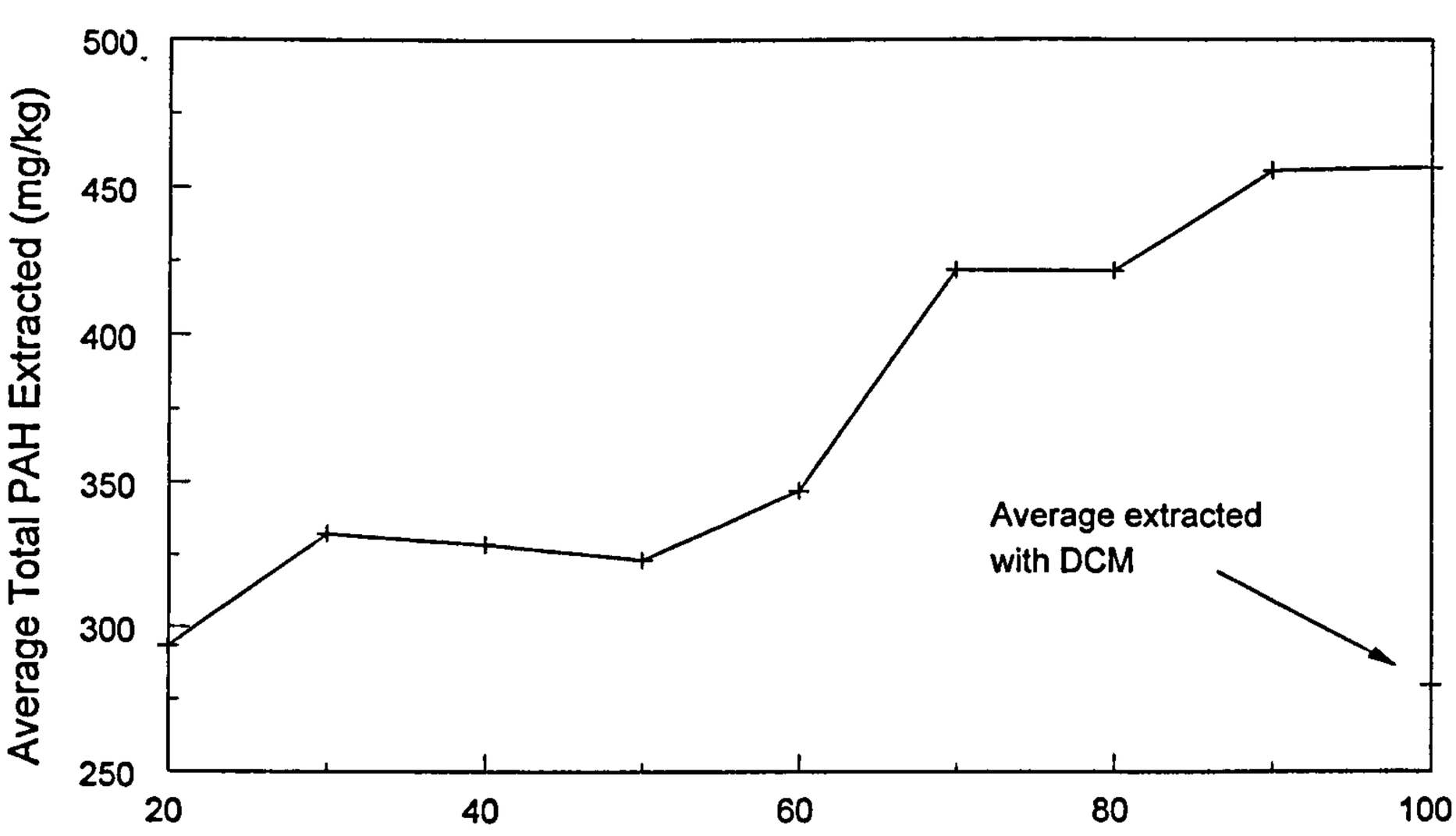


Figure 7.2 Average of Total PAHs Extracted as a Function of Percentage Acetone in a Acetone : Hexane Mixture.

To ensure any differences in detected PAH levels were solely due to an increased extraction amount and could not be attributed to GC injection anomalies when using

two different solvents (different transfer properties through the injector causing changes in the amounts of analytes to be transferred onto the column), a 10 μg ml^{-1} PAH standard, made up in both DCM and acetone, was injected into the GC split/splitless injector. The results showed no concentration variation (other than that associated with errors in making the two standards), which indicates that the increase in the amount extracted by the acetone is due to it being a more efficient solvent. Acetone may be a better solvent for extracting PAHs than, for example, hexane because of its permanent dipole which can cause dipole-induced dipole interactions with the numerous π-electrons present in the PAHs. Although hexane is non-polar, as are the PAHs, it is not possible for hexane to form dipole interactions with PAHs.

A repeatability study (summarized in table 7.3 and shown in full in table A5.8 in appendix 5), where pure acetone was used as solvent, was undertaken to ensure the validity of using 100 % acetone. All of the total PAH concentrations were found to be well over 400 mg kg^{-1}, with the relative standard deviation for individual PAHs being below 5 % with the exception of naphthalene and benzo (b + k) fluoranthene. The higher RSD for naphthalene may be explained because of the peak tailing that occurs which indicated the injection insert required replacement or from its loss upon opening the sample vessel after completion of an extraction (naphthalene is the most volatile PAH). It was also found that benzo (b) and (k) fluoranthene often gave poorer RSDs because of co-elution, illustrated in the chromatogram shown in figure 7.1.

Once acetone had been chosen as the most efficient microwave extraction solvent, a central composite experimental design was used to elucidate the optimum operating conditions for extracting PAHs. The three variables chosen for the study (temperature, extraction time and solvent volume) are all of primary concern when extracting using microwave energy. Pressure is another important variable but is directly dependent on the temperature of a particular solvent in a closed system and was therefore not specifically studied. Each of the variables was given upper and lower constraints which were normally set because of microwave operating limits (table 7.2). Due to the large number of experiments in the design, the experiments were performed on three consecutive days (blocks one to three). All experiments were carried out in duplicate with the results listed in table A5.9 in appendix 5. The experimental numbers in table A5.9 correspond to the different experimental conditions found in table A5.4. It is apparent from the results shown in table A5.9 that even though there is often a large variation in operating parameters between many of the experiments in the CCD, these differences do not greatly affect the concentration of PAHs recovered. Analysis of variance (ANOVA) was performed on the design to assess the significance of the

Compound	Average (mg kg^{-1})	% RSD
Naphthalene	12.0	5.4
Acenaphthylene	3.7	0.0
Acenaphthene	14.5	4.2
Fluorene	13.4	3.8
Phenanthrene	74.8	2.8
Anthracene	24.2	2.9
Fluoranthene	73.7	2.5
Pyrene	56.8	2.5
Benz(a)anthracene	26.3	3.3
Chrysene	27.3	2.5
Benzo(b)fluoranthene	19.8	5.3
Benzo(k)fluoranthene	18.3	5.7
Benzo(a)pyrene	24.2	3.8
Indeno(1,2,3-cd)pyrene	15.3	4.0
Dibenz(a,h)anthracene	3.7	0.0
Benzo(ghi)perylene	14.9	4.7
Total	422.9	2.4

Table 7.3 Summary Table of Repeatability Study (n = 6) Involving Microwave Extraction using 100 % Acetone (extracted at 120 °C for 20 minutes).

model, using the total concentration of PAHs extracted as the design response. The initial summary of the model statistics is shown in table 7.4.

Source	Sum of Squares	DF	Mean Square	F Value	Prob>F
Mean	3374639.9	1	3374639.9		
Blocks	5466.5	2	2733.3		
Linear	444.2	3	148.1	0.9134	0.4596
Quadratic	358.6	6	59.8	0.2502	0.9457
Cubic	1774.4	5	354.9	7.7950	0.0608
Residual	136.6	3	45.5		
Total	3382820.2	20			

Table 7.4 Summary of the ANOVA Model Statistics.

The F value shown in the table is the ratio between the mean-squared error (lack of fit of each model) and the pure error obtained from the replicates at the design centre. The significance of the F value, which is dependent on the number of degrees of freedom (DF) in the model, is shown in the final column in the table at the 95 % significance level. This *Prob*>F value represents the effect of adding the linear,

quadratic and cubic terms sequentially to the mean and block terms. The *Prob>F* values indicate that neither the linear or quadratic models are significant (< 0.05), with the cubic model the closest to the 0.05 significance level. However, it should be noted that with this design, the cubic model exhibits alaising (all of the model coefficients cannot be uniquely described) because of an insufficient number of experimental data points and therefore must be discarded. It is therefore concluded that none of the microwave operating variables chosen for optimization significantly affect the amount of PAHs recovered from soil 2. This is perhaps most simply shown by consideration of the relative standard deviation of the total PAH recoveries of all 20 experiments (and their duplicates) which is calculated to be 5.34 %. The relative standard deviation obtained when a repeatability study was performed using acetone as a solvent (table 7.3) shows parity. This suggests that any differences in recovery obtained during the CCD experiment were simply due to experimental uncertainty or caused by variations in the PAH concentration of the sub-sampled soil. For all subsequent extractions, the temperature and extraction time was set to their chosen upper limits, with the solvent volume maintained at 40 ml, since these conditions had already been shown to be successful in comparison with those reported by Soxhlet extraction.

The results from the CCD show that for a particular soil, simple microwave operating parameters may have little influence on the amount of analyte recovered. However, one other variable which was not studied in the experimental design was that of soil composition. The nature of the matrix in which the analytes of interest are bound can have a profound effect on the recovery of the compounds. This has been illustrated from spiking experiments, where solid samples are simply spiked with the analytes of interest, and compared with extractions from native soils. In almost all cases higher recoveries will be obtained from the spiked samples, demonstrating the effect of the stronger binding to the matrix in native samples.[88]

A third soil, of a different matrix composition, was extracted to observe any change in the recovery between Soxhlet and microwave extraction. A Laboratory of the Government Chemist (LGC) inter-laboratory test soil (CONTEST) was chosen for the study because the nature of the sample ensured that there would be little variation of PAH concentration between the whole sub-sample. The recoveries by Soxhlet extraction, discussed in section 7.3 (table 7.1), show excellent agreement with the microwave extraction recoveries (table A5.10, appendix 5) using both acetone and DCM as extracting solvents. The microwave operating conditions used were identical to those used to extract the other two soils. Again a large time saving (6 hours for Soxhlet extraction compared with 20 minutes microwave extraction) and a 50 %

reduction in solvent usage was possible using the microwave procedure. However, it is apparent from the results that there is no difference in the amount of PAHs recovered when using acetone instead of DCM as the extraction solvent. These results do not compare with the approximate 170 mg kg^{-1} increase in recovery found in the high concentration PAH soil (2), when the solvent type was changed. This result shows a variation in recovery when extracting different types of soil even under identical operating conditions and confirms that the recoveries obtained in microwave extraction (as with any other form of extraction) of contaminated land soils may be greatly affected by the nature of the soil matrix.

7.5 Supercritical Fluid Extraction

Supercritical fluid extraction has enjoyed much success for its organic solvent-free extractions using non-toxic carbon dioxide as the supercritical solvent. A great deal of work has been published on extraction of PAHs from soil using supercritical fluids, owing to the high solubility of the analytes in supercritical CO_2. However, despite the solubility of PAHs, their efficient recovery from environmental solids is greatly dependent on the nature of the matrix. Often organic modifiers are required in conjunction with CO_2 to effectively remove the PAHs,[163,166] which can vary substantially in molecular weight. Unfortunately, SFE has many other variables which can significantly affect analyte recovery and which are often poorly understood. These operating parameters may be best investigated, in the hope of optimizing them, using experimental design techniques which allow multi-variable systems to be investigated most efficiently. This approach therefore allows a reduction in the number of experiments required to fully understand a system and to ascertain the existence of any interactions between variables. This, in turn, can simplify the overall optimization procedure. The resultant procedure requires far fewer experiments to be carried out which are capable of yielding more information than the conventional "alter-one-factor-at-a-time" evaluation.

A central composite design, similar to the one used in section 7.4, is used to elucidate the optimum operating conditions for the removal of the maximum amount of PAHs from the contaminated land soil sample (soil 2). The combination of statistically designed experiments with the versatility of SFE allows a significant reduction in extraction time and method development. A second soil (LGC CONTEST) was also extracted by SFE to evaluate the effect of the matrix on recovery efficiency.

Procedure

Supercritical Fluid Extraction

All of the extractions were performed using the Jasco SFE instrument. The soil sample (1 g ± 0.01 g) was accurately weighed into a 4.6 mm i.d. stainless steel extraction cell, having a volume of 2.5 ml. The cell was placed inside the extraction oven, where it was allowed to equilibrate at the set temperature before commencement of extraction. Once the cell had reached the set temperature and pressure (the CO_2 flow-rate was kept constant at 1 ml min^{-1} throughout the experimentation), the pumps were switched off, and each sample was extracted for 5 minutes in static mode, prior to the dynamic extraction period. All extracts were collected by inserting the end of the back-pressure regulator through the PTFE-lined rubber septum of the modified collection unit which contained approximately 5 ml of DCM. The collection vial was placed in an ice-bath to reduce the amount of aerosol formation and thus, aid trapping. As before, at the end of the extraction, the C_{18} solid-phase extraction cartridge was back-flushed with 1 ml of methanol. The combined extract was made up to a final volume of 25 ml in DCM to await analysis.

As with the extracts obtained during microwave extraction (due to the smaller amount of sample used when compared with Soxhlet extraction), 500 µl of the extract was placed in a GC-MS autosampler vial together with 250 µl of internal standard solution to be analyzed.

Experimental Design

If we are to fully understand the way in which SFE operating variables affect an extraction there must be a consideration of the interaction between multiple variables and also non-linear effects. In this study, the variables which have been chosen are extraction pressure and temperature, time of dynamic extraction and percentage addition of methanol modifier. Methanol was chosen as the modifier because it has been shown to have the greatest effect on PAH extraction efficiency from native soils.[165] The increase in recovery from native samples is most likely due to matrix-modifier interaction (where the modifier can act in a competitive way releasing previously bound analyte) and/or the swelling of the matrix by the polar modifier. The flow-rate of CO_2 was kept constant throughout the experiments (1 ml min^{-1}) since it has been postulated that it does not affect extraction efficiency if the analytes are sufficiently soluble in the extracting fluid.[115] The four variables were studied using a full second-order central composite design (see section 3.1.4). Each of the variables had five separate coded levels: $-\alpha$, -1, 0, +1 and $+\alpha$ which required 30 experiments in

total. The design can be broken down into three groups of design points, a two-level factorial design (coded ± 1), "star" or axial points (coded ± α), and centre points (coded 0). The "star" points allow the estimation of the curvature in the model. In a four-factor (four variable) central composite design (CCD), the coded values of α are ± 2.00. The centre points allow the estimation of the "pure error" in the system. The results from the CCD can be fitted to a linear model (or response surface), which is adequate for describing a wide variety of multifactor chemical systems, of the form shown in equation 7.1.

$$Y_1 = \beta_0 + \sum_{i=1}^{q} \beta_i x_i + \sum_{i=1}^{q} \beta_{ii} x_i^2 + \sum_{i=1}^{q} \sum_{j=1}^{q} \beta_{ij} x_i x_j \qquad (7.1)$$

In this case Y_1 (the response) is the overall total amount of PAHs extracted and β_0 (the intercept) is the value of the fitted response at the centre of the design. Each variable was assigned set limits because of experimental and instrumental constraints. The pressure variable was given ranges between 100 - 300 kg cm^{-2} (x_1), temperature between 40 °C - 100 °C (x_2), extraction time between 10 - 60 minutes (x_3) and percentage methanol modifier between 0 and 20 % (x_4). The extraction time was fixed at a maximum of one hour to aid a relatively high sample throughput. These values were input into the statistical experimental design programme used to assess the microwave assisted extraction operating parameters (Design Expert), which used the variables to create a CCD matrix where the outer limits of the variables were used as the star points. The +1, -1 levels and the centre points were then calculated from these levels and used to complete the design. The final values for the design are shown in table 7.5.

Level	Pressure (kg cm^{-2})	Temperature (°C)	Ext. Time (min)	Methanol (%)
-α	100	40	10.0	0
-1	150	55	22.5	5
0	200	70	35.0	10
+1	250	85	47.5	15
+α	300	100	60.0	20

Table 7.5 The Values of the Four Variables (at 5 levels) used for the Central Composite Design.

The large number of experiments required for the design (30) necessitated that they be performed on three successive days, and the design was blocked into three sets of 10 experiments. The experimental blocking also has the advantage of allowing some assessment of reproducibility to be introduced into the model. The individual experiments in the blocks were then randomized to remove any systematic error, and the extractions were performed in that random order. Table A5.11 (appendix 5) shows the final full coded central composite design used in SFE optimization study.

Results and Discussion

Central Composite Design Results

The results of the central composite design are shown in full in appendix 5 (table A5.12). Each of the summed total PAH concentrations were used as the response (as opposed to the individual amounts) in the statistical evaluation. The reproducibility of the total PAHs extracted was determined to be 396.2 mg kg^{-1} (RSD 7.1 %), based on six repeat extractions over a three day period (using the centre points of the blocked experiments). Analysis of variance (ANOVA) was performed on the design to assess the significance of the model with the initial summary of the model statistics depicted in table 7.6.

Source	Sum of Squares	DF	Mean Square	F Value	Prob>F
Mean	2715321.7	1	2715321.7		
Blocks	33851.4	2	16925.7		
Linear	447599.3	4	111899.8	17.72	0.0001
Quadratic	100176.6	10	10017.7	2.89	0.0382
Cubic	26912.5	8	3364.1	0.93	0.5611
Residual	18142.0	5	3628.4		
Total	3342003.6	30			

Table 7.6 Summary of the ANOVA Model Statistics.

It should be noted that with the design, as with the design used in the microwave extraction optimization, the cubic model exhibits alaising (all β coefficients cannot be uniquely described) because of an insufficient number of experiments and should not be considered. As before, the *F* value in table 7.6 is the ratio between the mean-squared error and the pure error determined from the replicates at the design centre. The significance of the *F* value, which is dependent on the number of degrees of freedom (DF) in the model, is shown in the final column in the table (*Prob>F*) at the

95 % significance level and represents the effect of adding the linear, quadratic, and cubic terms sequentially to the mean and block terms. The summary statistics indicate that the quadratic model should be further investigated since the quadratic terms are significant (< 0.05) when added to the mean and linear terms of the model.

The *lack of fit* of each model is summarized in table 7.7 and indicates how well each of the full models fits the experimental data. This is achieved by comparing the residual error to the pure error from the replicated design points. The *Prob>F* value in the final column of the table again indicates any significant (95 %) *lack of fit* of each model.

Model	Sum of Squares	DF	Mean Square	F Value	Prob>F
Linear	142353.8	20	7117.7	7.421	0.0618
Quadratic	42177.2	10	4217.7	4.398	0.1248
Cubic	15264.7	2	7632.4	7.958	0.0632
Pure Error	2877.3	3	959.1		

Table 7.7 Summary of the Lack of Fit of the Models.

In this case, non of the models show any significant lack of fit to the experimental data points, at the 95 % confidence level, but the quadratic model exhibits the least significant lack of fit. The quadratic model was then fully evaluated using ANOVA to determine which variables, if any, had a significant effect on total PAH recovery. This was achieved by dividing each coefficient by its associated standard error to obtain a Student's value *t*, which tests whether the coefficient is different from zero, the null hypothesis. The associated *p* values (Prob>$|t|$) are interpreted as the probability of getting a coefficient as large as that observed when the true coefficient equals zero. Therefore, small values of p (< 0.05 at the 95 % significance level) indicate significant terms in the model. The significance test results are shown in full in table 7.8.

The results show that there are only three significant variables which affect total PAH recovery: extraction time (x_3), percent methanol modifier (x_4), and the squared term of modifier addition (x_4^2). By far the most statistically significantly of these coefficients are the methanol modifier terms. No other coefficients were found to have an effect on PAH extraction efficiency, and the extremely low significance of the cross-product terms suggests no interactions between variables is likely. The final second-order polynomial in terms of actual values was calculated using the software and is shown in equation 7.2.

Independent Variable	Coefficient Estimate	Standard Error	"*t*" for null hypothesis	Prob> |*t*|
Intercept	396.2	24.0	16.490	
Block 1	-27.1			
Block 2	-20.2			
Block 3	47.3			
x_1 [a]	18.7	12.0	1.555	0.1440
x_2 [b]	15.9	12.0	1.320	0.2097
x_3 [c]	**27.5**	**12.0**	**2.290**	**0.0394**
x_4 [d]	**131.5**	**12.0**	**10.940**	**0.0001**
x_1^2	-22.1	11.2	-1.970	0.0705
x_2^2	-18.0	11.2	-1.606	0.1324
x_3^2	-22.5	11.2	-1.998	0.0671
x_4^2	**-56.6**	**11.2**	**-5.033**	**0.0002**
x_1x_2	-1.6	14.7	-0.111	0.9131
x_1x_3	-2.9	14.7	-0.195	0.8488
x_1x_4	5.7	14.7	0.389	0.7036
x_2x_3	0.5	14.7	$3.57e^{-2}$	0.9721
x_2x_4	-1.0	14.7	$-7.05e^{-2}$	0.9449
x_3x_4	4.1	14.7	0.278	0.7856

Table 7.8 Results of Significance Test on Quadratic Model Standardised Coefficients (significant values are shown in bold).

[a] Pressure (kg cm^{-2}); [b] Temperature (°C); [c] Extraction Time (min); [d] Methanol Content (%).

$$\textbf{Total PAH} = -1239.1 + 4.0015\,P + 12.764\,T + 12.330\,Ti + 65.658\,\%MeOH - 8.859e^{-3}\,P^2 - 8.021e^{-2}\,T^2 - 0.1438\,Ti^2 - 2.269\,\%MeOH^2 - 2.183e^{-3}\,PT - 4.580e^{-3}\,PTi + 2.290e^{-2}\,P\%MeOH + 2.800e^{-3}\,TTi - 1.383e^{-2}\,T\%MeOH + 6.54e^{-2}\,Ti\% \quad (7.2)$$

Where: Pressure = P, Temperature = T, Extraction Time = Ti and
Percent Methanol = %MeOH.

The effect of the two significant variables (percent modifier and extraction time) on the overall total PAH recovery is illustrated with a response surface (figure 7.3). Obviously, only two independent variables can be visualized using a 3-D response surface, with the third axis as the dependent response. The other two variables in the

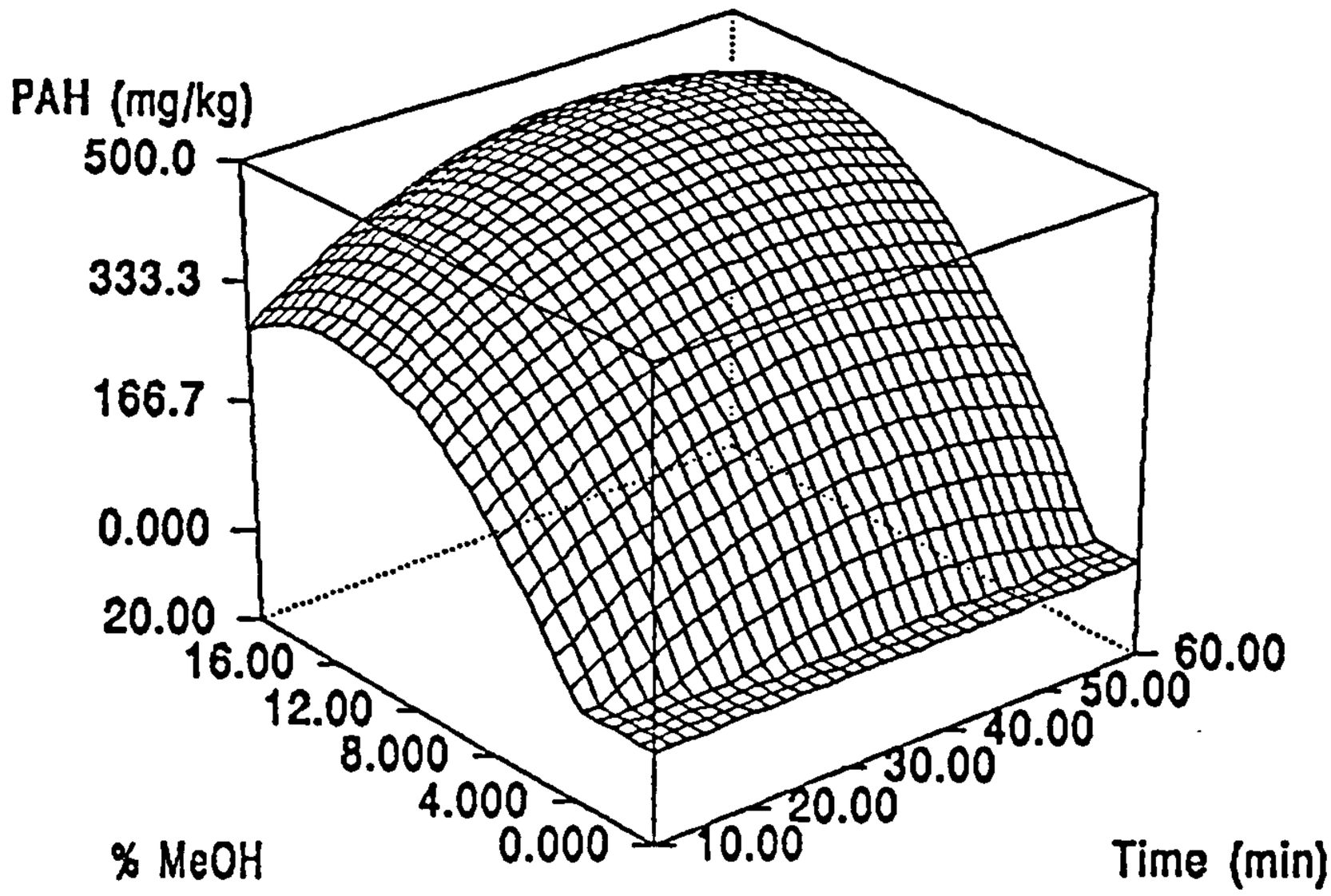


Figure 7.3 Response Surface for PAH Recovery from Contaminated Land Samples (soil 2) Showing the Effect of Percent Methanol Modifier and Extraction Time on the Extraction Efficiency at 200 kg cm^{-2} and 70 °C.

design were kept constant at their mid-point values. The surface graphically shows that percent modifier has, by far, the greatest influence on PAH recovery.

An analysis of the residuals (actual values *versus* those predicted by the model) was performed to reveal any inherent problems with the ANOVA model. The analysis included a normal probability plot (normal probability *versus* studentized residuals), a plot of student residuals *versus* the predicted response and two other residual plots (studentized residuals *versus* both run number and a specified factor, in this case percent methanol) designed to check for any systematic errors in the experiment.

In all cases the diagnostic plots indicated that the chosen quadratic model fit the data correctly. The four diagnostic plots are shown in appendix 5 in figures A5.1a-d.

The results from the central composite design were then used to predict the SFE operating conditions which would result in the most efficient extraction of PAHs from the contaminated land sample. The final model indicated that both the pressure and temperature used in the extraction had no statistical significance on the PAH recovery. Consequently, both parameters were kept at their mid-point values (200 kg cm^{-2} and 70 °C, respectively) for the optimized extraction. The remaining two variables (extraction time and percent methanol modifier) were operated at their maximum

values since the model predicted that both had a direct influence on the total PAH recovery. The final optimum conditions chosen were: pressure, 200 kg cm^{-2}; temperature, 70 °C; extraction time 60 minutes; and methanol content 20 %. These conditions were used in a repeatability study to assess the effectiveness of the models optimization. The results of the study, shown in appendix 5 (table A5.13), show an overall average recovery of 458.0 mg kg^{-1} (RSD 3.1 %, $n = 7$). This recovery is very similar to the maximum obtained during the design experiments (run 24 in table A5.12), under comparable conditions (with the exception of percent modifier which is only 10 %), indicating the correct operating conditions. In addition, the precision is slightly better than that obtained during the CCD since the centre repeats were extracted over three separate days. Sequential repeat extractions were also carried out on the samples from the first three experiments in the repeatability study to investigate whether the initial extractions were exhaustive. If possible, in sequentially extracting samples, it is important to increase the strength of the second extraction to test the efficiency of the first. Unfortunately, it was not possible to increase the percent methanol added to the sample because of the detrimental effect of the polar solvent has on gas chromatographic analysis with non-polar columns. It would also not be practical to increase the time of extraction if still desiring a high sample throughput. Therefore the sequential extractions were performed under identical operating conditions as the first. GC-MS analysis failed to detect PAHs present in any of the second extracts, which indicated that the first extraction was exhaustive, in terms of SFE, under the optimized operating conditions.

The results of the optimized SFE study may be compared with those obtained from a six hour Soxhlet extraction and the average obtained using microwave assisted extraction (using acetone) which are 297.4 mg kg^{-1} (RSD 10.0 %) and 422.9 mg kg^{-1} (RSD 2.4 %), respectively. The average PAH concentration extracted using SFE compares favourably with that obtained using microwave energy and is far greater than the amount extracted by Soxhlet, although it should be noted that no attempt was made to optimize the Soxhlet extraction operating conditions. The comparison between the three extraction techniques is discussed further in section 7.6.

Extraction and Analysis of the CONTEST Soil Sample

An inter-laboratory test sample, containing a similar concentration of PAHs as the previous sample, was also used to test the optimized SFE conditions. The soil was extracted under identical conditions as those already used. However, during the dynamic extraction period, the CO_2 pump periodically reached its pressure maximum with the back-pressure regulator reading as normal. This indicated some blockage in

the system, possibly by co-extractives. Each time this occurred, the blockage was cleared by flushing the entire system with solvent and a new extraction performed. Upon analysis by GC-MS in total ion mode, large amounts of elemental sulphur were found in the chromatogram, in much higher concentrations than the extracted PAHs, which may account for the system blockage. A typical chromatogram during the extraction of the LGC soil (3) by methanol-modified CO_2 is shown in appendix 5 (figure A5.2). The presence of high concentrations of sulphur in the soil has also been discussed in section 7.3 when yellow crystals were observed on the glass surface of the flask after the completion of the Soxhlet extractions involving soil 3. Problems with elemental sulphur are well known, and samples containing percent levels of sulphur have been successfully extracted by SFE by using copper as a scavenger to remove the sulphur before it is allowed to cause blockages or interfere with chromatographic detection.[126,162] The results of the repeatability study are listed in appendix 5 (table A5.14) and were obtained when the instrument did not block. The results show quite widely varying total PAH concentrations with associated high RSD values. The precision compares poorly with the results from Soxhlet and microwave assisted extractions which recovered 242.7 and 244.6 mg kg^{-1}, respectively, although on average, the overall amount extracted using SFE is higher (280.9 mg kg^{-1}).

7.6 Soxhlet, Microwave Assisted Extraction, and Supercritical Fluid Extraction of Polycyclic Aromatic Hydrocarbons: A Comparison

Soxhlet, microwave assisted extraction, and supercritical fluid extraction have been evaluated for the removal of PAHs from contaminated land samples, through sections 7.3-7.5. A summary of the overall recoveries and an indication of the method precision, for all three extraction techniques are shown in tables 7.9a and 7.9b, for soil 2 and soil 3, respectively.

Extraction Technique	Solvent Used	Recovery (mg kg^{-1})	Number of Extractions	% RSD
Soxhlet*	DCM	297.4	5	10.0
Microwave*	DCM	279.8	4	13.0
Microwave	Acetone	422.9	6	2.4
SFE	CO_2 + MeOH	458.0	7	3.1

Table 7.9a Summary of Extraction Recovery Data for Soil 2.

Extraction Technique	Solvent Used	Recovery (mg kg^{-1})	Number of Extractions	% RSD
Soxhlet*	DCM	242.7	3	1.0
Microwave*	DCM	247.5	2	-
Microwave	Acetone	241.7	2	-
SFE	CO_2 + MeOH	280.9	4	14.0

Table 7.9b Summary of Extraction Recovery Data for Soil 3 (CONTEST).

* Conditions and solvent not optimized

Conditions for both microwave assisted extraction and SFE were optimized using a central composite design prior to analysis of the soil. The results indicate that (a) the choice of solvent is critical in order to maximize PAH recovery, (b) microwave assisted extraction and SFE are efficient at recovering native PAHs from the contaminated soils studied, and (c) the nature of the soil matrix may greatly affect the efficiency of the extraction. Based solely on these results, the choice of extraction technique would seem to be between microwave assisted extraction and SFE, even though Soxhlet extraction is thought to yield the "correct" result (note that no attempt was made to optimize the Soxhlet extraction procedure and that the method used is routine). However, prior to recommending the most appropriate extraction technique for PAHs from soil, there are several other factors which require consideration (table 7.10). Each of the factors will now be discussed.

Sample Mass

The mass of sample used in the extraction plays an important role if the level of each contaminant approaches the sensitivity of the analytical instrument used for quantitation, as occurred with soil 1. However, with the other two soils, the level of contamination was so high that instrumental sensitivity was not a particular problem. Therefore for samples containing low concentrations of PAHs, the benefit of using 10.0 g of sample in Soxhlet extractions is obvious. This advantage can be negated with the use of solvent evaporation techniques and/or solid-phase extraction pre-concentration, although the processes have inherent disadvantages including possible losses of more volatile analytes and the additional time taken for each analysis. In the comparison study, an initial attempt to use a greater amount of soil (5.0 g) in microwave assisted extraction was abandoned due to the occurrence of electrical arcing within the sample vessel. No similar occurrence was evident when the sample mass was reduced to 2.0 g. No attempt was made to increase the soil mass used in the

	Soxhlet Extraction	Microwave Assisted Extraction	Supercritical Fluid Extraction
Sample mass	10.0 g	2.0 g	1.0 g
Extraction time	6 hours	20 min (plus 30 min for cooling and pressure reduction)	1 hour (plus 10-15 min for temp. / press. equilibration)
Sequential or simultaneous operation	Six separate assemblies used	up to 12 vessels can be used simultaneously	sequential in the system used
Equipment cost	low (< £1 000)	high (~£15-20 000)	highest (£15-40 000)
Operator skill	low	medium	high
Organic solvent volume used for extraction	100 ml of DCM	40 ml (acetone / DCM)	CO_2, 12 ml MeOH and 5 ml DCM for collection

Table 7.10 Additional Factors for Consideration for the Extraction of PAHs from Contaminated Soils.

SFE study, but it should be noted that commercial extraction cells for analytical SFE are available with sample volumes up to 100 ml.

Extraction Time

The major advantage of microwave assisted extraction when compared to more conventional solvent extraction techniques (*i.e.* Soxhlet) is the speed of extraction. However, in all of the work detailed in section 7.4, the sample vessels remained un-opened until the pressure within the control vessel reached atmospheric pressure (approximately 30 minutes) to reduce the possibility of losing the more volatile PAHs, such as naphthalene. For SFE, an extraction time of 65 minutes (5 minutes static plus 60 minutes dynamic) and an additional 10-15 minutes sample equilibration time was required in order to achieve the high recoveries reported. The 6 hour Soxhlet extraction period was not investigated further since it was a standard method used in the collaborating environmental analytical laboratory and the overall aim of the project was to investigate sample preparative techniques which reduced solvent usage.

Sequential or Simultaneous Operation

For Soxhlet extraction, each sample was extracted using an individual assembly but the low cost nature of the technique allows multiple assemblies to be used.

Unfortunately, one disadvantage of this is the large volume of laboratory space the Soxhlet apparatus consumes which are required to be used in a fume hood. The use of the commercial microwave instrument allows the extraction of up to 12 samples simultaneously and occupies far less laboratory space and only the exhaust tube requires the use of a fume hood (the microwave unit can be operated outside). In each case, one sample vessel also contains pressure and temperature sensory equipment. Disadvantages of extracting many samples simultaneously include an increased time required to heat the samples from room temperature to the pre-set extraction temperature which is directly dependent on the number of samples being extracted (as well as the solvent used). In addition, when extracting multiple samples an assumption is made that each individual sample is behaving in an identical manner to the one contained in the sensory vessel, which may not be the true case. In this study, the SFE system used was only capable of extracting sequentially. However, other commercial SFE systems allow either automated or unattended operation as well as simultaneous (up to 8 samples) extraction.

Equipment Cost

Soxhlet is by far the lowest cost alternative in terms of instrumental price. However, with increased pressure to reduce organic solvent usage and ever increasing disposal costs and the cost of labour, combined with the high degree of automation, reliability and efficient recovery possible using the more modern techniques, failure to investigate both SFE and microwave assisted extraction as viable alternatives to Soxhlet, based solely on cost, is not justified.

Operator Skill

One of the reasons why Soxhlet is still so widely used is for the extraction of organic pollutants from soils is its simplicity of use. The degree of operator skill required for the other two techniques in the comparative study is graded with the highest operator skill, based on the current commercial instrumentation, being required for SFE. Any move towards creating more fully automated SFE systems which can be run using "recipe-type" procedures will provide a stronger case for its overall acceptance. While some steps toward this goal have been made by certain SFE instrument manufacturers, their exuberant cost often makes them prohibitive for routine use. Alternatively, with microwave assisted extraction, operator skill is somewhat reduced because of the common use of microwave appliances in the home.

Organic Solvent Usage

Any reduction in the volume of organic solvents, particularly chlorinated, required per analysis is advantageous. The ability of SFE to use non-toxic carbon dioxide and therefore significantly reduce the need for organic solvents (may be completely eliminated if on-line SFE is used) is often quoted and has been used as a major selling point of SFE instruments. In this study, the lower amount of solvent used in SFE is noted, as well as the higher amounts required for both microwave and Soxhlet extractions (although the amount of solvent used in microwave assisted extraction is approximately half that used in Soxhlet extraction).

An additional comparison between the effects of the different solvent compositions used in the optimum operating conditions of the three techniques is illustrated by the typical GC-MS total ion chromatograms (TIC) obtained for Soxhlet (figure 7.4a), microwave assisted extraction (figure 7.4b), and SFE (figure 7.4c). The peaks in the chromatograms labelled 1-16 correspond to the PAHs under investigation in the order in which they appear in all results tables. It is evident from chromatograms (a) and (c) that both Soxhlet and SFE, respectively, show similar levels of extractives, principally PAHs. However trace (b), the microwave assisted extraction TIC, contains additional peaks giving higher responses than the target PAHs. The additional major extracted components were tentatively identified, using a mass spectral database contained on the GC-MS software, to be complex phenols which may have been preferentially extracted using the acetone solvent because of their polar nature. Less polar DCM (Soxhlet) or CO_2-methanol (SFE) may have failed to remove these polar interferences. One other explanation for the presence of the phenol interferences in the microwave extracts is that microwave energy may have begun to breakdown the soil leading to the unwanted peaks (although they were not seen during routine analysis since selected ion monitoring was used throughout). Further comparison to the effectiveness of microwave assisted extraction to SFE is shown in figure 7.5 which shows a photograph of an extract derived from SFE with CO_2 only, SFE with methanol-modified CO_2, and a microwave extract using acetone. The more intense colour of the right-hand extract is additional evidence of the non-selective nature of this technique. Conversely, this non-selective nature may be advantageous in extracting a wide variety of analytes, such as those required in an EPA semi-volatile analysis suite.[340] The inability of SFE, using non-polar CO_2, to effectively solubilize more polar analytes may exclude it from consideration in similar analysis.

In conclusion, the merits and disadvantages of each extraction technique have been discussed. Both microwave assisted extraction and SFE are shown to be feasible

alternatives to traditional solvent extraction Soxhlet techniques. Unfortunately, it is not clear which sample preparative technique is most suitable since non are perfect and each have inherent problems. In fact in many instances, the role of the sample matrix can have the most profound effect on analyte recovery. However, with modern environmental pressure to reduce the consumption of hazardous organic solvents any procedure which does so will become more prominent. It is clear that with a better understanding of the principles of extraction, techniques can be modified or re-designed to improve the reported extraction efficiency.

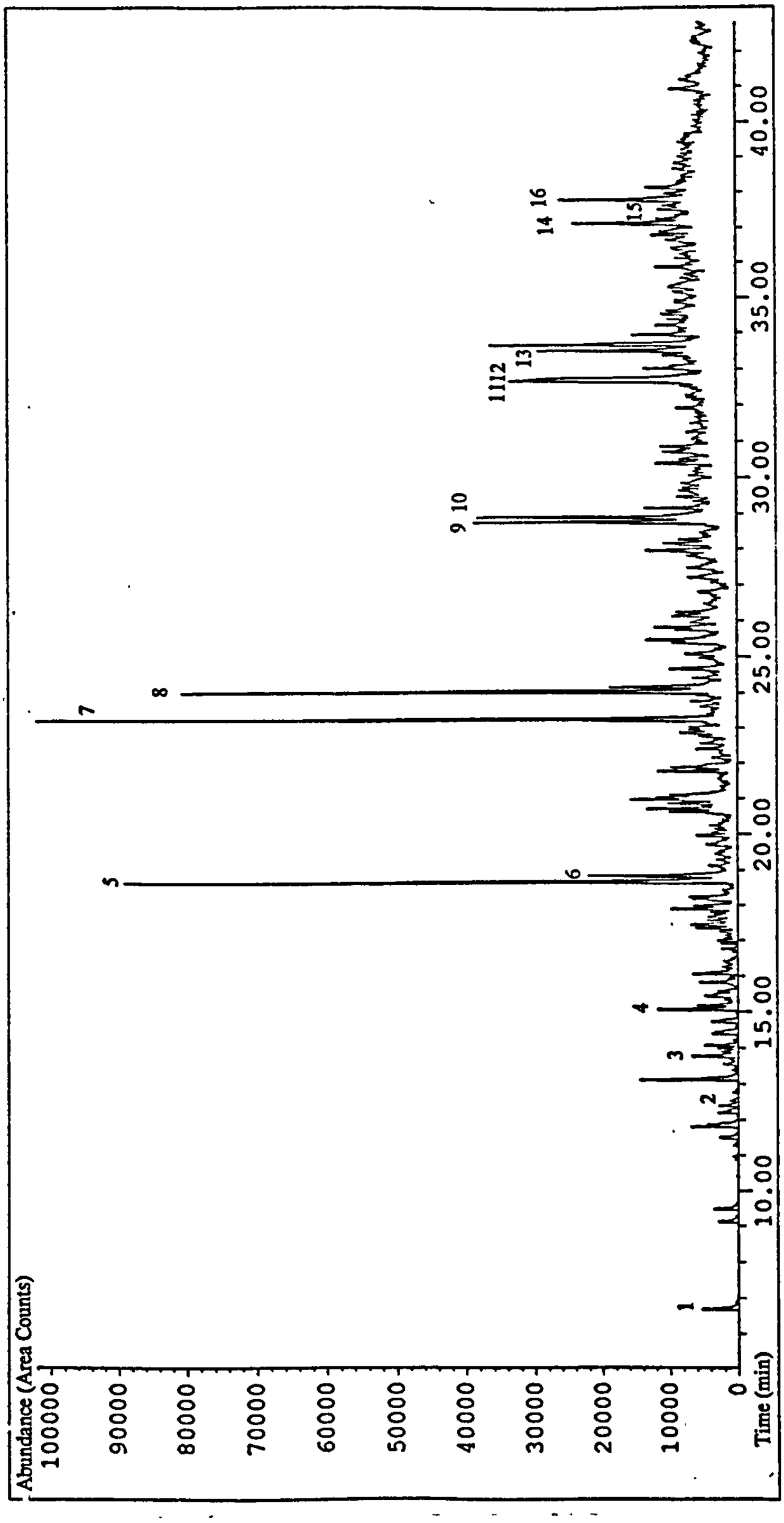


Figure 7.4a Total Ion GC-MS Chromatogram of the Extract Obtained by Soxhlet Extraction (DCM) of Soil 2.

Peaks 1-16 represent the PAHs investigated in the study which elute in the order in which they appear in the results tables.

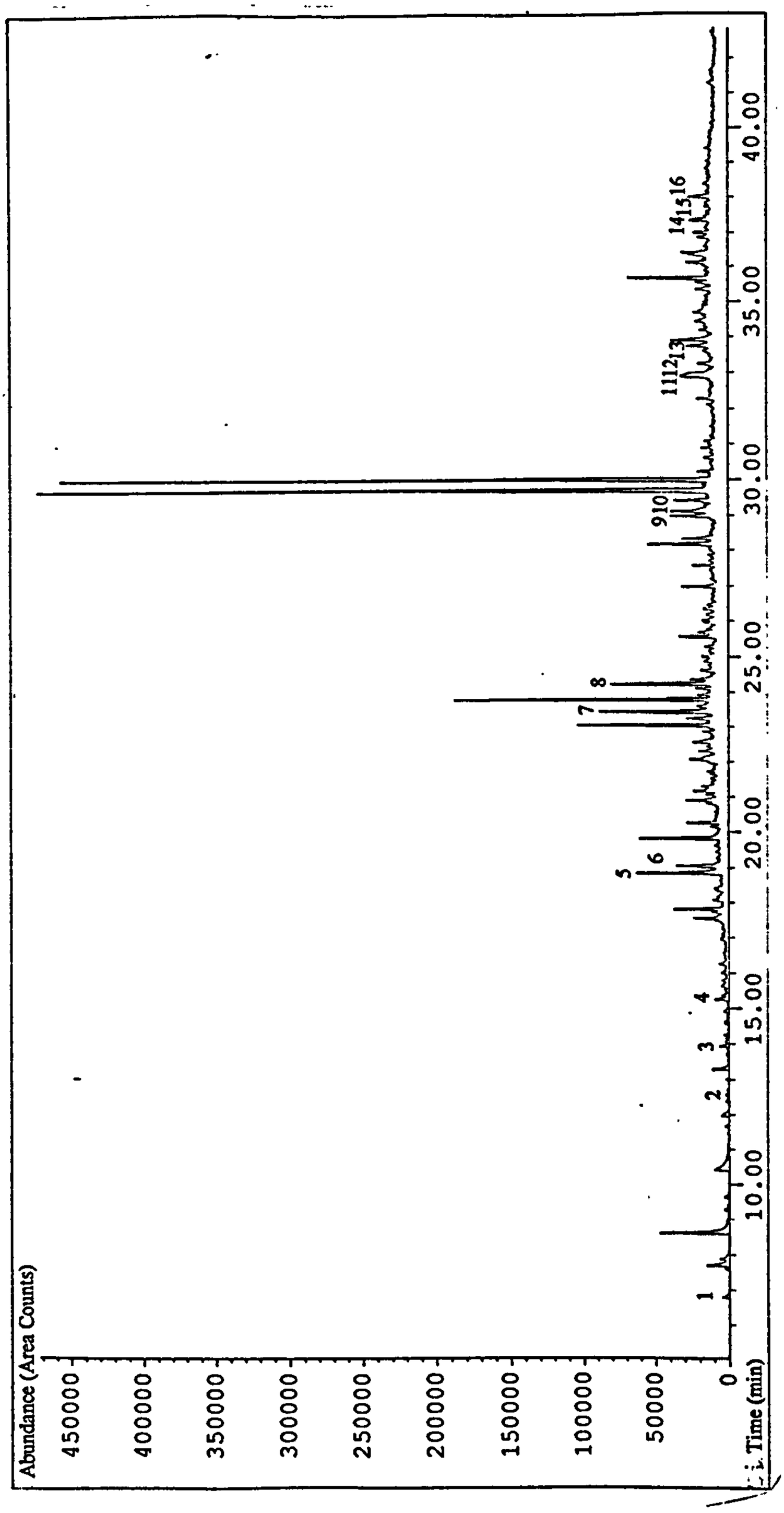


Figure 7.4b Total Ion GC-MS Chromatogram of the Extract Obtained by Microwave Assisted Extraction (Acetone) of Soil 2.

Peaks 1-16 represent the PAHs investigated in the study which elute in the order in which they appear in the results tables.

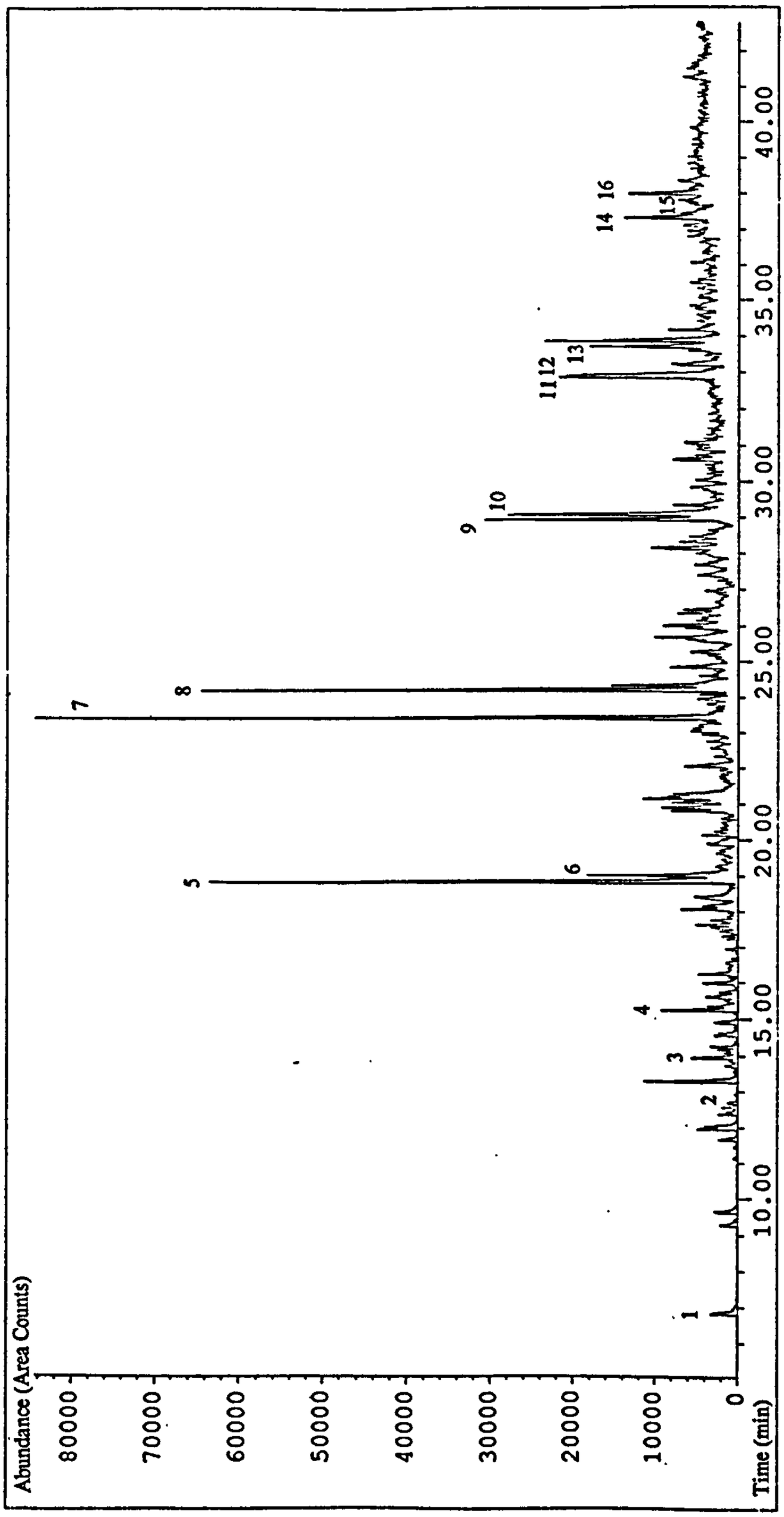


Figure 7.4c Total Ion GC-MS Chromatogram of the Extract Obtained by Supercritical Fluid Extraction (CO_2 + Methanol) of Soil 2.

Peaks 1-16 represent the PAHs investigated in the study which elute in the order in which they appear in the results tables.

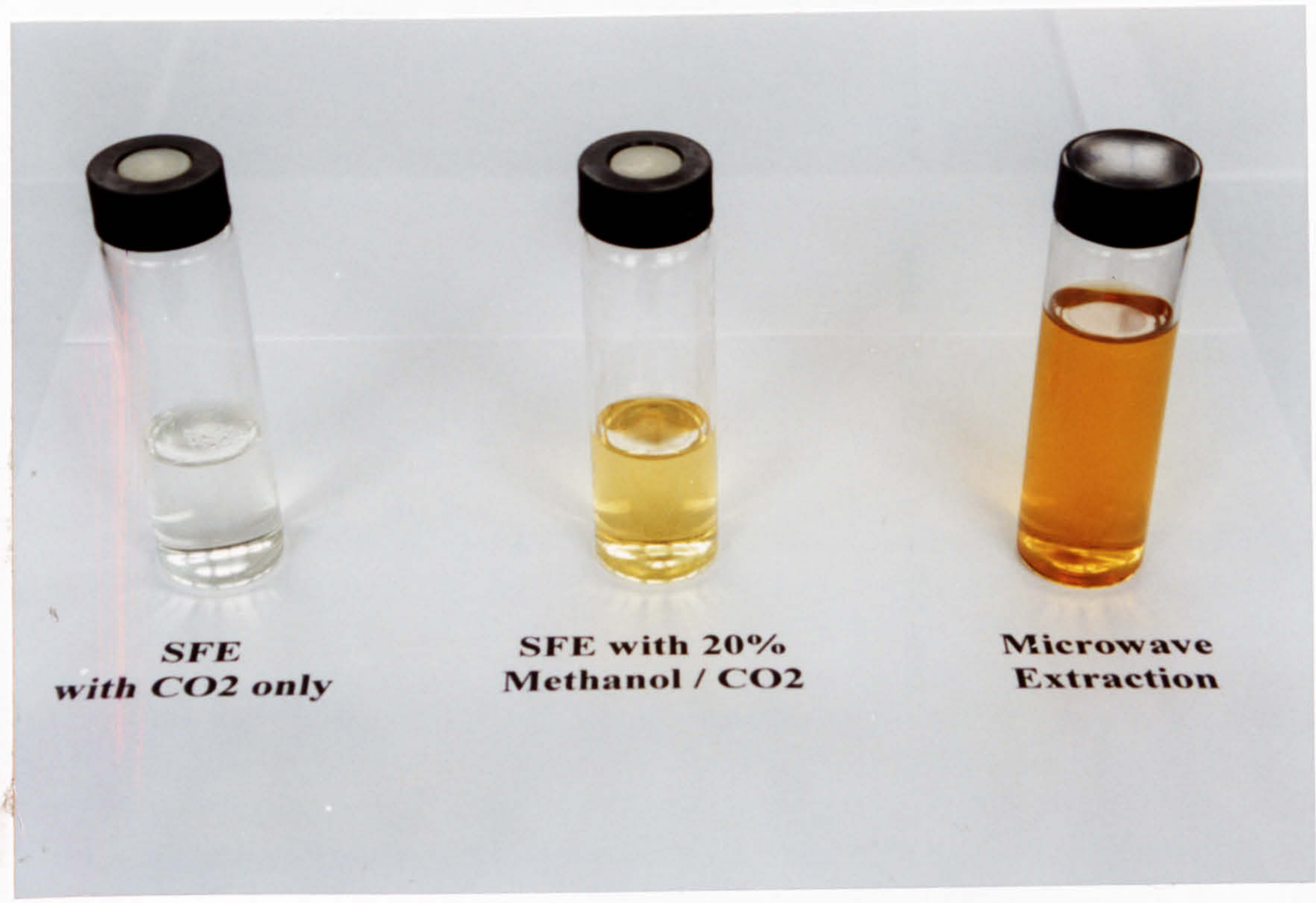


Figure 7.5 Photograph of Supercritical Fluid (Left and Middle) and Microwave Assisted Extraction (Right) of Contaminated Soil (2).

Chapter 8

Influence of Pesticide-Soil Interactions on the Recovery of Pesticides using Supercritical Fluid Extraction

8.1 Influence of Pesticide-Soil Interactions on the Recovery of Pesticides using Supercritical Fluid Extraction

Supercritical fluid extraction has been used extensively to recover organic pollutants from solid samples, and in particular soils. However despite its popularity, less than quantitative recovery is frequently achieved, despite having often obtained acceptable extraction efficiencies during initial investigations where the analytes are extracted from inert matrices. While this phenomena is not peculiar to SFE, it does indicate that poor analyte solubility in the extracting fluid is not the primary cause of poor recovery. The low extraction efficiency is principally attributed to strong binding of the analytes onto the soil which cannot be removed with supercritical CO_2 and / or modified CO_2, although there is little evidence to account for this.

This chapter investigates the correlation between soil properties and recovery of a range of pesticides (including herbicides) from spiked soils using SFE. The recoveries are compared to those obtained from an inert matrix (Celite).

8.2 Supercritical Fluid Extraction of Selected Pesticides from Characterized Soils

Perhaps the most difficult factor to understand when determining the variables which affect extraction efficiency in any sample preparative technique is that of the matrix. This is particularly so for solid samples as this type of matrix often displays the greatest sample to sample variation even if acquired from the same site. In the previous chapter, it was observed that on one particular soil (2), the choice of extraction solvent had a significant effect on the total concentration of PAHs recovered, whereas on a second soil (3), no difference in the amount extracted was noted. This indicates that in this case, the soil matrix has a pronounced effect on the recovery of PAHs.

The strong retention of neutral organic analytes by soils has been linked, in many investigations, to the percentage of organic matter present in the soil. However, in the majority of SFE publications, this is the only soil variable which is associated with compound sorption.[129,146,148] This is perhaps an over-simplification since many other factors may contribute to overall retention including analyte structure and other soil properties. Many studies have considered the successful extraction of pesticides from soil.[108,116,117,124] Unfortunately, due to the lack of availability of "real" soil

samples, many of these publications use convenient laboratory "spot" spiking techniques in order to obtain representative samples.[108,117] This method of introducing compounds onto solid samples simply involves injecting a solvent solution of the analytes onto the soil surface. Unfortunately, laboratory prepared samples frequently do not mimic the effect of natural analyte "ageing" in soil. This is particularly true using spot spiking regimes, since the compounds of interest are not brought into intimate contact with the majority of the sample. If, due to the poor availability of native samples, laboratory prepared samples must be used, a more rigorous spiking procedure is that of "slurry" spiking. Here the analytes are prepared in a solvent solution which is thoroughly mixed with the sample to ensure all of the surface area of the sample comes into contact with the analytes. After this, the solvent is allowed to evaporate with care being taken to prevent losses of the more volatile compounds. Recent studies have compared the two techniques and found that whilst quantitative recovery was obtained using a spot spiking protocol, a significant reduction in extraction efficiency was observed when the analytes were slurry spiked onto identical matrices and was similar to that obtained from a native soil sample.[89,139]

In this section, the effect of the soil matrix in SFE is investigated further using previously characterized soils to observe the effect different soils have on the recovery efficiency of slurry spiked pesticides and herbicides. The analytes chosen represent a large cross-section of routinely analyzed pesticides, with a wide structural functionality, and were those used previously in the research (with the exception of chlorfenvinphos, an organophosphorus pesticide). The classes include organochlorine pesticides, organophosphorus pesticides, and both s-triazine and urea herbicides. Unfortunately, native soils containing the pesticides of interest were not available and therefore laboratory prepared samples were used throughout the study. The different classes of analytes were individually slurry spiked onto four characterized soils representing a wide range of organic matter contents, since it is this soil variable which is most often linked with strong compound retention. SFE was then used to remove the compounds under similar conditions as those used previously to successfully extract the same analytes from water. The recoveries were compared with those obtained from an "inert" matrix (Celite) to assess the significance of the soil matrix.

Procedure

Soil / Celite Spiking

Once the pesticides and herbicides had been chosen for the study, a series of experiments were performed to evaluate the applicability of the spiking protocol. This involved spiking the Celite with solvent and initially determining the time period required to completely remove all traces of solvent. This was achieved by weighing the vessel containing the Celite / solvent mixture until no difference in weight was observed. 1 g ± 0.1 g of each individual soil (or Celite) was accurately weighed into a vial and 5 ml of DCM added. Each pesticide group (OCPs, OPPs, and triazine / urea herbicides) was then spiked into the soil / solvent slurry at the 10 μg level and mixed using a small stirbar on a magnetic stirrer for 10 minutes. The slurry was then left at room temperature (23 ± 2 °C) in a fume hood to allow the solvent to evaporate. From this study, it was determined that all of the DCM was removed in under 10 hours at room temperature. It was therefore decided, for simplicity, to leave the slurry mixture for a period of approximately 24 hours to remove the solvent, so as not to act as a solvent modifier during SFE.[341] Having completed this initial study, the analytes were evaporated at elevated temperatures (50 °C) to assess whether their volatility meant that they may be lost during evaporation. Having spiked the analytes in the Celite / solvent slurry, the solvent was evaporated in an oven for 30 minutes. The study revealed that none of the analytes, with the exception of dichlorvos (see results and discussion section), were removed when evaporated at this increased temperature, and it was therefore assumed that room temperature evaporation of the solvent ensured complete retention of the pesticides.

Once the evaporation stage had been completed, the spiked soil was transferred into an extraction cell and any difference in weight from that initially taken, recorded and used to correct the recovery obtained from each extraction (assuming uniform distribution of pesticides in the soil). Each type of soil used was subjected to characterization for percentage organic matter, quartz, clay, aluminium and iron content, specific gravity and cation exchange capacity (CEC).[342] The complete characterization of the soils (including those used in the batch distribution coefficient study, discussed later) is tabulated in appendix 6, table A6.1.

Supercritical Fluid Extraction

All extractions were performed using the Jasco SFE system described in chapter 4, fitted with the modified collection unit. The dried soil / Celite was carefully transferred into the extraction cell and the weight difference in the vial noted. After

temperature equilibration, all samples were extracted at 250 kg cm^{-2} and 50 °C (a CO_2 density of 0.85 g ml^{-1}), using a 15 minute static extraction period followed by a minimum of a 30 minute dynamic extraction (40 minutes was deemed necessary for extraction of OCPs). In the case of the OPPs and herbicides, a methanol modifier was used to obtain adequate extraction efficiency. A CO_2 flow-rate of 2 ml min^{-1} was used for the extraction of both OCPs and herbicides, although could not be used for the removal of OPPs due to the high volume of methanol modifier which would have been produced in the collection vessel being incompatible with gas chromatographic analysis using non-polar columns. The flow-rate used to extract OPPs was therefore reduced to 1 ml min^{-1}. Under all conditions, an ice-bath was also used to cool the solvent in the collection vessel in an attempt to reduce the amount of aerosol formation caused by the depressurization of the CO_2.

Analysis of the extracts was performed by either GC-MS (organochlorine and organophosphorus pesticides) or HPLC (herbicides), with the conditions described in chapter 4 (sections 4.8 and 4.9, respectively).

Results and Discussion

Initial Studies from Celite

Initial work sought to investigate the optimum conditions for the extraction of the pesticides from an inert solid matrix, *i.e.* Celite. In each situation, each class of pesticide or herbicide was assayed with respect to optimum extraction efficiency using either supercritical CO_2 only or methanol-modified CO_2. The conditions used were similar to those used previously to extract the same classes of analytes from aqueous matrices and therefore a methanol modifier was required to successfully remove both the OPPs and herbicides but was not needed for the extraction of the relatively non-polar OCPs. Once chosen, the extraction conditions were used to extract previously spiked Celite five times in a repeatability study in order to obtain an average recovery for the individual analytes. The results of the study are summarized in table 8.1 (which also lists the appropriate extraction conditions) and given in full in appendix 6, tables A6.2 - A6.4. Overall, the results show good precision with a relative standard deviation for the repeat extractions of no worse than 8.4 % for any compound.

Typical GC-MS chromatograms for both OCPs and OPPs are shown in figures 8.1 and 8.2, respectively (both at the 1 μg ml^{-1} concentration level). Note HPLC

Pesticide Type	Pesticide	Extraction Conditions	Average Recovery (%)
Organochlorine	Lindane	1*	94.5
Organochlorine	Aldrin	1	87.6
Organochlorine	Dieldrin	1	90.4
Organochlorine	Heptachlor	1	95.4
Organochlorine	Isodrin	1	89.8
Organophosphorus	Diazinon	2**	97.8
Organophosphorus	Malathion	2	87.6
Organophosphorus	Chlorfenvinphos	2	93.4
Triazine Herbicide	Simazine	3***	81.6
Triazine Herbicide	Propazine	3	80.3
Triazine Herbicide	Trietazine	3	91.7
Urea Herbicide	Chlortoluron	3	80.4
Urea Herbicide	Isoproturon	3	80.0
Urea Herbicide	Diuron	3	77.0

Table 8.1 Summary of Average Recovery from Celite ($n = 5$).

* (1) 250 kg cm^{-2}; 50 °C; 2 ml min^{-1}; 15 minutes static; 40 minutes dynamic.

** (2) 250 kg cm^{-2}; 50 °C; 1 ml min^{-1}; 15 minutes static; 30 minutes dynamic; 5 % methanol.

*** (3) 250 kg cm^{-2}; 50 °C; 2 ml min^{-1}; 15 minutes static; 30 minutes dynamic; 10 % methanol.

chromatograms illustrating the analysis of the herbicides used in the study are identical to those shown in section 6.3 and are therefore not shown.

It is observed that the mean extraction efficiency is acceptable in terms of quantitative extraction from an inert matrix for each OCP studied. In particular, unlike the majority of the work carried out using combined SPE-SFE, dieldrin is extracted with equal efficiency when compared to the other compounds in its class.

For the OPPs, their moderate polarity required the addition of methanol-modified supercritical CO_2. The results indicate that quantitative extraction recovery was achieved for the three OPPs studied using 5 % methanol-CO_2. In this case it should be noted that chlorfenvinphos, a routinely analyzed pesticide, was preferred over dichlorvos which was used in all of the previous studies involving OPPs. This was

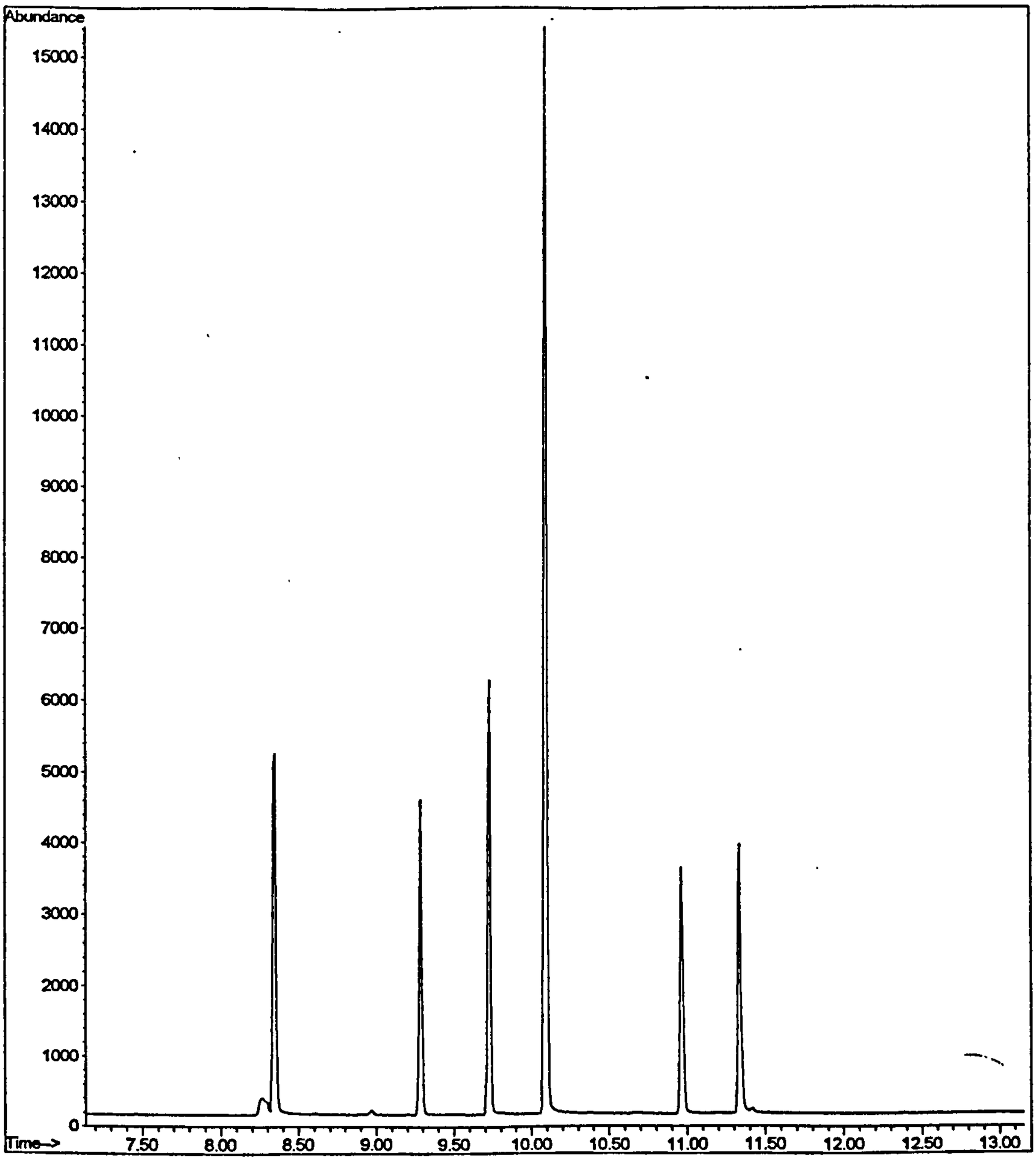


Figure 8.1 A Typical GC-MS Chromatogram for OCPs used in the Soil Study.
Elution Order: lindane, heptachlor, aldrin, isodrin, dieldrin and β-endosulphan.

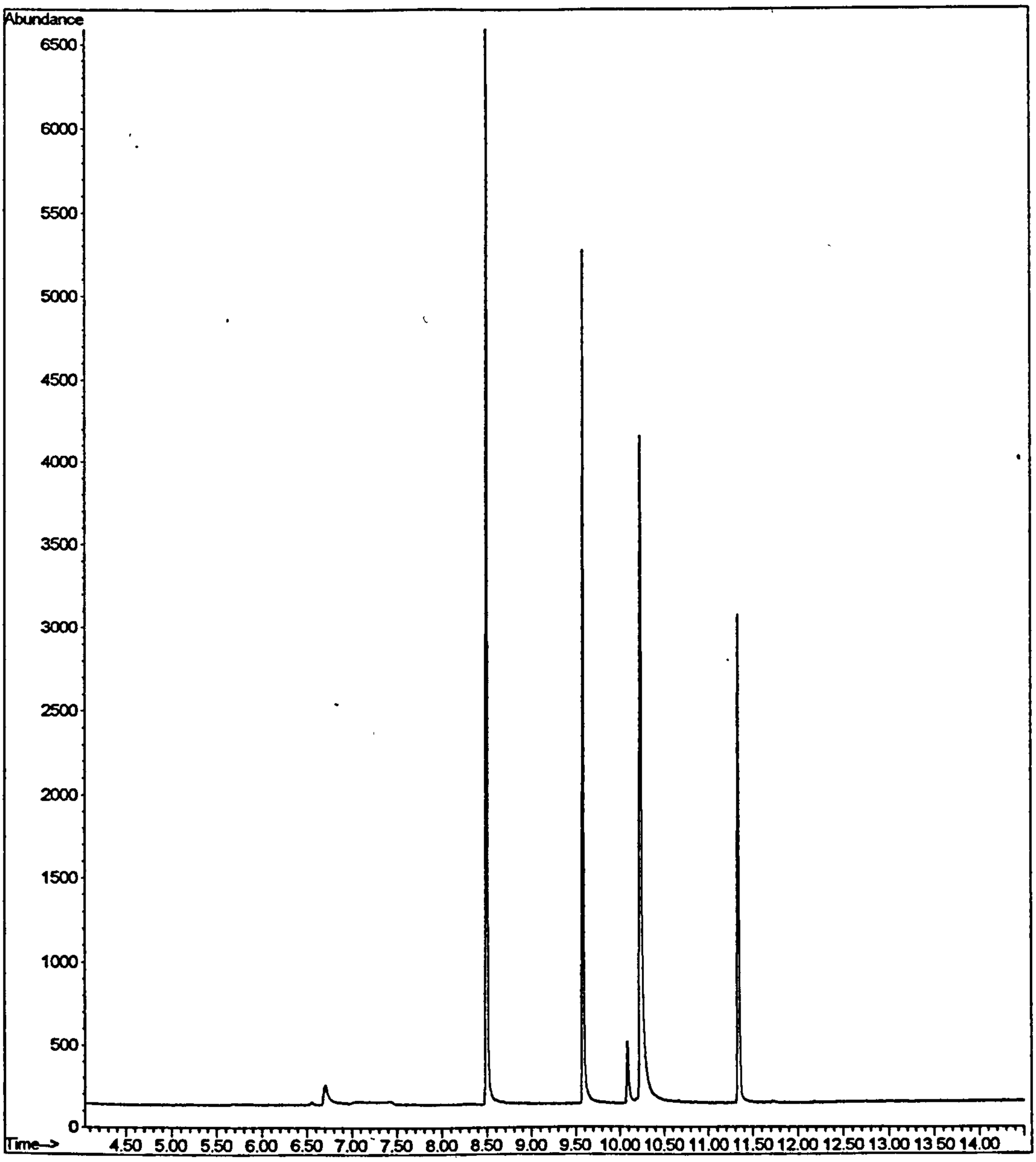


Figure 8.2 A Typical GC-MS Chromatogram for OPPs used in the Soil Study.
Elution Order: diazinon, malathion, chlorfenvinphos and β-endosulphan.

due to the high volatility of dichlorvos which prevented it from being successfully spiked onto the Celite without almost complete loss upon evaporation of the solvent (even at room temperature). The high volatility of dichlorvos is illustrated by reference to its vapour pressure of $2.2x10^{-3}$ mmHg (at 20 °C) when compared to the other OPPs (chlorfenvinphos, diazinon, and malathion) which have vapour pressures of $7.5x10^{-6}$ mmHg (25 °C), $1.4x10^{-4}$ mmHg (20 °C), and $4.0x10^{-5}$ mmHg (30 °C), respectively.[343] The high vapour pressure of dichlorvos is mirrored by its use as a fumigant pesticide.[25] The in-volatile nature of chlorfenvinphos (used as a foliage applied dust or liquid) make it an ideal replacement for dichlorvos, since the development of spiking regimes for volatile compounds was not the intended purpose of the study.

The optimum recovery of the relatively polar herbicides was achieved using 10 % methanol-modified supercritical CO_2. However, even the addition of this relatively high amount of polar modifier did not allow quantitative recovery of the herbicides. The poor extraction efficiency of the herbicides may be due to the nature of the "inert" matrix chosen for the initial study. Celite, or diatomaceous earth is obtained naturally and is formed by the silicaphilation of prehistoric plankton and is principally used as an inert support because of its homogeneous nature and large pore structure and surface area. However, it is perhaps inappropriate to refer to the solid as "inert", since its silica structure offers a high affinity for polar molecules due to its large surface area which contains many uncapped silanol groups capable of hydrogen bonding with polar molecules. Indeed, it appears that the worst recovery of all the pesticides studied occurs when the molecule contains an amide functional group (urea herbicides), carboxylate groups (malathion), or a high proportion of nitrogen's in the molecule (triazine herbicides), all of which are capable of hydrogen bonding with the numerous silanol groups in Celite.

Extraction from Spiked Soils

Each soil used in the study was obtained from the same site at increasing depth from the surface and characterized for a range of physical and chemical properties. A total of twelve air dried soils were available although only four were chosen as they represented the widest variety in organic content, a property known to be associated with strong organic analyte retention. The soils chosen (at levels L5, L7, L8, and L11 in appendix 6, table A6.1) had organic content values which ranged from 0.2 % to 35.0 %. This provides a diverse representation of extreme soil types likely to be found in the environment. The low organic matter type soil is characteristic of sand while the high organic content is typical of a peat-based soil. Each class of pesticide was

extracted in at least duplicate. The spiking protocol and SFE conditions employed were identical to those used when extraction from Celite was performed. The average results for the OCPs are shown in table 8.2 (in full in table A6.5) in order of increasing percentage organic content (with Celite results for comparison).

% Organic	Soil No.	Lindane	Aldrin	Dieldrin	Heptachlor	Isodrin
-	Celite	94.5	87.6	90.4	95.4	89.8
0.2	L5	52.0	65.7	44.5	51.6	44.8
2.0	L11	50.1	58.8	30.8	54.8	36.5
15.0	L8	51.9	69.9	51.0	57.5	49.0
35.0	L7	30.2	42.8	30.8	36.3	30.4

Table 8.2 Summary of Average Percentage Recoveries of Organochlorine Pesticides.
Conditions: 250 kg cm^{-2}; 50 °C; 2 ml min^{-1}; 15 minutes static; 40 minutes dynamic.

It is observed that even the soil containing the lowest percentage organic matter has a dramatic effect on extraction recovery. The average recovery from each of the five pesticides investigated was 51.7 % from the 0.2 % organic matter soil when compared to 91.5 % extracted from Celite. No significant difference was observed between the other soil-types until that is the soil with an organic matter content of 35 % was investigated. In this situation, it is observed that the mean recovery of the five pesticides has decreased to 34.1 %, a reduction of almost 60 % compared to that obtained from Celite.

For the OPPs, the situation is different. It is observed (table 8.3, and in full in table A6.6) that within experimental error, no significant difference in recovery is noted between the recoveries from Celite and the different soil types, once again the exception being the 35 % organic matter content soil. The average recovery of the three OPPs investigated from this soil decreased to 44.7 %, which is a decrease of approximately 50 % compared to the recovery from both Celite and the other soils.

% Organic	Soil No.	Diazinon	Malathion	Chlorfenvinphos
-	Celite	97.8	87.6	93.4
0.2	L5	84.5	54.4	57.2
2.0	L11	93.6	94.6	104.4
15.0	L8	96.7	76.3	79.0
35.0	L7	43.6	42.9	47.7

Table 8.3 Summary of Average Percentage Recoveries of Organophosphorus Pesticides.
Conditions: 250 kg cm^{-2}; 50 °C; 2 ml min^{-1}; 5 % Methanol; 15 minutes static; 30 minutes dynamic.

The average percentage recoveries for the two classes of herbicides are shown in table 8.4 and in full in table A6.7 (appendix 6).

% Organic	Soil No.	Simazine	Propazine	Trietazine	Chlortoluron	Isoproturon	Diuron
-	Celite	81.6	80.3	91.7	80.4	80.0	77.0
0.2	L5	61.0	62.4	69.6	60.6	59.2	58.3
2.0	L11	64.6	59.4	58.3	58.3	55.5	47.5
15.0	L8	75.5	71.9	72.6	67.3	78.6	61.5
35.0	L7	73.3	71.1	59.7	67.7	64.0	61.5

Table 8.4 Summary of Average Percentage Recoveries of Both s-Triazine and Urea Herbicides.

Conditions: 250 kg cm^{-2}; 50 °C; 2 ml min^{-1}; 10 % Methanol; 15 minutes static; 30 minutes dynamic.

The SFE of the two classes of herbicides was affected by the presence of the soil matrix, but to a lesser extent than that observed with the OCPs. In this case, the average amount extracted from Celite is approximately 82 % and the minimum average extracted is observed from soil L11 to be approximately 57 %, a reduction of only 25 %. However, the individual variation within each average value prevented any noticeable influence that the soil may have induced from being shown. It was also surprising to note that no effect was seen for the extraction recovery from the soil with the high organic content, with all soil types offering a similar degree of retention of the herbicides. This may be due to the inorganic fraction of the soil competing for the retention of these relatively polar compounds, thus reducing the significance of the organic fraction. This theory was recently proposed as the mechanism behind triazine sorption in soils containing small to medium levels of organic matter.[37]

In general therefore, it can be inferred that the non-polar OCPs appear to be influenced by the soil organic matter irrespective of the nature of the soil. The ability of the pure supercritical CO_2 to extract OCPs from soil is limited, which implies a stronger interaction between the soil and the OCPs than with supercritical CO_2. However, the apparent poor extraction efficiency demonstrated using the SFE conditions chosen may be due to their excellent performance during the initial Celite extractions used to determine the operating conditions. In this case, Celite may truly be acting as an "inert" matrix for the very non-polar OCPs which therefore require relatively mild conditions to successfully extract them. This in turn may lead to inappropriate operating conditions being chosen for extraction from soil and cause the analytes to appear more difficult to extract from the soil matrix.

The addition of 5 % methanol-modified supercritical CO_2 is able to quantitatively extract the moderately polar OPPs from both Celite and soil. Here the greater solvent strength afforded by the modifier compensates for the increased difficulty in removing the analytes from the soil matrix compared to Celite. In the case of malathion, Celite appears to cause retention to a similar extent as the soils. However, when the organic matter of the soil is at its highest (35 %) the situation becomes less favourable for the methanol-modified CO_2 and retention of OPPs occurs.

In contrast, the use of 10 % methanol-modified supercritical CO_2 appears to provide a suitably polar solvent to extract the two classes of herbicide from the soil, irrespective of the organic matter content. In an opposite effect to that of the OCPs, Celite does not offer a suitable inert support to allow initial operating conditions to be determined and within experimental error behaves in a similar manner to each of the soils.

Overall, with the exception of the polar herbicides, the extraction efficiency of the analytes studied appears to be most influenced by percentage organic matter in the soils investigated. No other soil property evaluated (other than cation exchange capacity which shows a direct relationship with percent organic matter) influences the retention behaviour of the pesticides studied. Unfortunately, a statistical study proved difficult due to the large variance of the replicate data derived from the soil study. This may have been due to the inhomogeneous nature of the soil when compared to that of Celite.

An identical relationship has been reported during an investigation of pesticide / soil batch distribution coefficients (Kd) utilizing the same soils.[344] In this study, a range of ^{14}C (beta emitter) radiolabelled pesticides and herbicides (including lindane, atrazine, and isoproturon) were spiked into a buffered aqueous solution and gently agitated with each soil (in a fixed soil / volume ratio) for one week. A large constant excess of non-radioactive analyte was also present in each mixing vessel to account for discrepancies in ^{14}C-labelled concentration of the individual pesticides and to allow pesticide Kd values to be compared. In addition to the 12 soils studied, a control sample containing only the buffer solution, non-labelled and ^{14}C-labelled pesticide, was also mixed. Once mixing was complete, the soil / buffer slurry was centrifuged and a set volume of supernatant removed for liquid scintillation counting. The difference in counts between the control sample and the sample containing the different soils was then used to determine the amount of labelled pesticide bound to the soil.

In all of the experimental studies, the amount of pesticide bound to the soil was found to have a direct relationship with the percentage organic matter, *i.e.* the concentration of soil-bound pesticide was found to increase proportionally with the soil organic content. None of the other soil parameters were statistically found to have any significant influence on pesticide sorption. The influence of soil organic matter has also been strongly associated with strong retention in many other studies.[34,39,345] Following multiple linear regression, a quadratic function was found to best fit the relationship between organic matter content and Kd, with correlation coefficients ranging between 0.90 and 0.99. This quadratic relationship has also been found for lindane on soils of differing organic matter contents.[35]

In conclusion, prediction of the extraction conditions required to successfully remove organic contaminants from a soil matrix is much more unpredictable than removal of organics from aqueous samples or non-polar solid-phase extraction media. In fact, the extraction conditions used previously to quantitatively remove the same analytes from water appear inadequate when the pesticides are spiked onto soils. It is therefore obvious that despite the lack of native samples, slurry spiking techniques offer a viable alternative to assess sample preparation efficiency when "real" samples are unavailable. Also, Celite may not be a suitable indicator to assess the extraction efficiency of SFE conditions as it cannot be regarded as an inert matrix for polar compounds. Additionally for highly non-polar analytes, extraction from Celite underestimates the solvent strength required to quantitatively extract analytes from a real matrix.

In general, the extraction of pesticides from soil can be influenced by the polarity of the supercritical fluid. This influence is most noticeable when the organic matter of the soil is high. This dramatic decrease in recovery between moderate and low organic matter content soil is most noticeable of the OCPs and OPPs. To negate the influence from the soil organic matter content, as in the case for the herbicides, it would appear to be advantageous to use a highly polar supercritical fluid *i.e.* by using a relatively high modifier content.

Achievement of Initial Aims

Achievement of Initial Aims

In general, the overall aim of the project was to investigate sample preparation techniques which were capable of extracting organic environmental pollutants from both solid and liquid matrices, with a mandate to significantly reduce the consumption of organic solvents. Throughout the work, the project has allowed the majority of the initial aims to be achieved using several modern analytical techniques. Supercritical fluid extraction (SFE) using carbon dioxide predominated in much of the research and was used to extract both aqueous and solid samples. The technique was shown to be inadequate in removing pesticides (of great concern as possible contaminants in drinking water) directly from aqueous samples, although was capable of quantitative recovery when combined with solid-phase extraction (SPE). In addition, SFE was shown to allow some selectivity in extraction to be performed by alteration of extraction conditions. In all cases where SFE was utilized, solvent consumption (relative to liquid-liquid or Soxhlet extraction) was greatly reduced because of the use of non-toxic carbon dioxide. Also SFE has the ability to be fully automated (although not in the actual system used) and therefore warrants further investigation as a high-throughput sample preparative technique. Unfortunately, SFE did not allow a reduction in the overall extraction time for liquid samples because of the need for a SFE-SPE combined stage.

As well as SFE and SPE, solid-phase microextraction (SPME) has also been utilized to remove herbicides from aqueous samples. This technique has been shown to meet all of the projects overall aims as it allows fully automated extraction (and analysis) to be performed more rapidly than standard liquid-liquid partitioning. However, perhaps the most significant aspect of SPME is its ability to completely eliminate solvent usage and perform solvent-free extractions.

In the second half of the project, solid samples (soils) have also been extracted using instrumental sample preparation methods. Again SFE has predominated and has been used to remove polycyclic aromatic hydrocarbons (PAHs) from different types of soil. This class of compound was chosen because of its ubiquitous presence in the environment and hence its inclusion in the majority of contaminated land studies. In addition to extraction using supercritical fluids, organic solvent extraction using microwave energy has also been assessed for its ability to extract PAHs for the same matrix. Both techniques were shown to have their own advantages however in general, as before, SFE used less solvent and microwave assisted extraction was far faster than Soxhlet extraction. As with SFE, microwave assisted extraction is capable

of being automated and in addition can process up to twelve samples simultaneously thus greatly increasing laboratory sample throughput.

In conclusion, all instrumental sample preparation techniques have been shown to reduce solvent consumption (in the case of SPME, to completely eliminate it) whilst still quantitatively extracting the analytes under investigation. This is a general requirement as stricter environmental policies on solvent consumption may prohibit usage, particularly of chlorinated solvents. SFE, SPME, and microwave assisted extraction all show the ability to be partly or even fully automated and therefore offer great potential benefits to a high sample throughput analytical laboratory. Finally, the majority of the techniques studied, and in particular those associated with soil analysis, have indicated that a significant reduction in extraction time may be feasible without any detrimental effects to the quality of the extraction procedure. This in turn shows great potential to save analysis time and thus increase the overall volume of samples capable of being analyzed.

Chapter 9

Conclusions and Suggestions for Future Work

Throughout the project, many organic sample preparation techniques have been investigated which utilize a wide variety of instrumentation. All of the instrumental protocols used were chosen because they are capable of quantitatively extracting organic analytes from complex environmental matrices, whilst significantly reducing laboratory solvent consumption and simultaneously increasing sample throughput, primarily through a greater scope for automation. These techniques are generally used at the beginning of an analysis protocol and their recent progression is paralleled by the advances in instrumental chromatographic separation techniques routinely used to quantify the extracts they produce. The majority of the techniques studied are relatively new (<10-15 years old), if not in their inception, then in their application to organic analysis and therefore must warrant further investigation into their possible roles in organic sample preparation.

Supercritical fluid extraction has been used extensively throughout the research, principally because of the reported success of supercritical fluids in extracting environmental solids. However, the technique was first used in the project to extract pesticides from aqueous samples, both directly and indirectly using a solid-phase extraction protocol. Direct extraction of the test analytes (organochlorine pesticides) proved difficult and was largely unsuccessful, primarily because of slow diffusion kinetics through the aqueous matrix and the solubility of water in supercritical carbon dioxide. Despite this, the method could potentially be used as a qualitative screening technique although with the advent of solid-phase extraction and solid-phase microextraction, the author cannot foresee the need to investigate the use of SFE using carbon dioxide for direct extraction from aqueous samples further.

Far greater success was attained when SFE was used in conjunction with SPE to extract the same analytes from water. The advantages of the combined technique compared to the direct extraction were essentially that all traces of water could be removed prior to supercritical elution and since the pesticides were no longer in the aqueous matrix, diffusion kinetics were not significant. Near quantitative recovery was obtained for all the OCPs (with the exception of lindane) using SPE-SFE although unfortunately, the combined technique proved lengthy and it is difficult to envisage a commercial application of SPE-SFE for single component (or class of components) from aqueous samples.

Despite the time-consuming procedure, further work was carried out using SPE-SFE to investigate the possibility of the selective extraction of pesticides from water. This area was investigated as the controlled extraction of analytes, based on their physical

and chemical properties, may be of great use in certain analytical areas (*i.e.* where different detection systems are used). The ability to change the solvating power of supercritical fluids by altering their density is well documented, although few examples of this phenomenon are seen in literature. The organochlorine pesticides used in the previous studies were again used as test analytes and were selectively extracted from both organophosphorus pesticides and two classes of herbicides. The selectivity achieved was obtained due to differences in the analytes polarity and the use of low and high density CO_2, together with the addition of a polar solvent modifier (methanol). In the case of the herbicides, near complete selectivity was obtained from the OCPs, although the fractionation of OPPs proved more demanding. However, it is difficult to predict whether similar results could be as easily obtained if conventional liquid solvents had been used to elute the analytes.

In all of the studies involving supercritical fluid extraction from aqueous samples, OCPs were used as test analytes. In the final work, where OCPs were selectively extracted from herbicides, alternative pesticides were chosen from the same class with the exception of dieldrin which was retained to observe its extraction behaviour. In the three separate studies involving the extraction of dieldrin from water using a combined SPE-SFE approach, there appears to be a significant difference in the recovery data obtained. In the first study (extraction of OCPs only) the average recovery of dieldrin is 91.6 %, whilst in the two selective extraction studies, average recoveries of 72.0 % and 84.8 % are reported for the selective extraction of OCPs from OPPs and herbicides, respectively. Surprisingly, this difference in recovery appears to be directly correlated with the concentration of dieldrin spiked into the water prior to adsorption onto the SPE disk. This in turn was dependent of the sensitivity of the chromatographic technique used to analyze the extracts. In the first example a pesticide level of 10 μg was used, however, for the next two studies, levels of 200 μg and 100 μg were used, respectively. Thus the higher the concentration of dieldrin in the aqueous samples, the poorer the recovery obtained.

Future work may be of use in exploring this theory since the exact reason for the difference in recovery is not known. However as stated previously, this is unlikely to be due to retention of the epoxide ring of dieldrin by the uncapped silanol groups in the disk. This is indicated since greater than 90 % of dieldrin is recovered from Celite in chapter 8 (a matrix which also contains a high proportion of silanol groups). Therefore the poor recovery of this organochlorine pesticide may be due to break-through of the compound during SPE caused by a relatively strong affinity for water when compared to lindane and aldrin, both of which do not contain oxygen

functionality capable of hydrogen bonding. Further work in this area could be directed towards evaluating this theory by using other OCPs which have a similar structure to dieldrin, to determine whether their behaviour in aqueous media is similar. An example of this could be the use of isodrin which is epoxidized to endrin (as aldrin is to dieldrin), which could be compared in similar studies involving the SPE-SFE of all four compounds.

As a general comment regarding the concentration of the extracted analytes, elevated levels of pesticides and herbicides have been used throughout the SFE studies when compared to those conventionally analyzed in water quality compliance laboratories. Typical maximum allowable concentrations of pesticides in drinking water are around 0.1 $\mu g\ l^{-1}$ and are detected using one litre samples. This corresponds to a spiking level of 0.02 μg in the 200 ml sample used in the research. However in the SPE-SFE studies performed, levels as high as 200 μg were utilized to assess the performance of the technique and it is impossible to say whether the recoveries obtained at this vastly different level are a true reflection on those which would have been derived from studies at the concentrations routinely analyzed in drinking water. The use of these elevated levels is solely due to the chromatographic instrumentation available at the time of the work which was incapable of obtaining the detection limits required for water analysis. Having stated this, the overall aim of the work was not to investigate the SPE procedure (as many publications are concerned with break-through volumes in SPE) but instead to explore the possibility of using supercritical fluids to elute pesticides from solid-phase extraction media. However, it may be the intention of future work to re-evaluate the extraction procedures using concentrations which are more representative of those encountered in environmental laboratories. To achieve the desired low detection limits, such work would require an increase in the volume of sample extracted, a reduction in the final extract volume by solvent evaporation and an overall increase in the sensitivity of the chromatographic instrumentation used for analysis.

In addition to the work undertaken to investigate the extraction of pesticides and herbicides from water using supercritical fluids, a new development in the form of solid-phase microextraction (SPME) was also investigated for its applicability to extract and analyze s-triazine herbicides in the same matrix. In the majority of previous research involving SPME technology, the focus has been directed towards volatile organic compounds such as BTEX, since these are favourably partitioned in the headspace above the aqueous sample and are easily desorbed at relatively low temperatures. However, the class of herbicides studied can be termed semi-volatile

compounds and are solids under normal conditions. This work therefore demonstrates the application of SPME to a whole new class of compounds and is of particular interest because of its ability to allow fully automated extraction and analysis, at a fraction of the cost of conventional SPE, with the complete elimination of organic solvents.

Several different operating parameters were investigated in the study, including different fibre coatings and adsorption / desorption times and temperatures, with the overall aim to achieve detection limits which were applicable to drinking water analysis. Unfortunately, initial experimentation using a nitrogen-phosphorus GC detector indicated that this would not be feasible. However with further work, the possibility of performing multiple adsorptions from the same sample was investigated and was found to allow detection limits in the region of those of use to the water analysis laboratories.

Further work in the area necessitates the use of a more sensitive GC detector, for example an ion-trap MS system (currently used in many of the recent publications on SPME). This combined with the ability to perform multiple extractions will allow detection limits far below those already obtained using a NPD system. This in turn would therefore allow smaller sample volumes of water to be analyzed whilst still allowing the required detection limits to be achieved.

At the present time, the one main disadvantage to the automated SPME system is its inability to allow some form of sample agitation during the adsorption stage. This would greatly enhance the overall performance of any fibre and is essential if solid coatings are used as adsorbent phases (as in the case of the polyacrylate fibre). Sample agitation would allow the time taken for adsorption to be greatly reduced and simultaneously enhance the precision of the analysis since the procedure would no longer be solely dependent on thermal diffusion through the aqueous matrix. Future work using SPME which is to be applicable to routine analysis must involve sample agitation in order to reduce overall analysis time and its reliance on thermal diffusion. At the present time the company responsible for the manufacture of automated SPME hardware is currently performing tests on a prototype system which allows the actual fibre itself to be agitated in the matrix whilst adsorption is taking place. Any future work would benefit from this novel system.

SPME, although still in its infancy as an environmental extraction technique, has indicated sufficient promise to ensure its place for routine analysis of aqueous

matrices. The advent of new commercially available fibres with a range of specific analyte coatings will promote its use in environmental laboratories of the future.

In the second half of the thesis, SFE was compared against traditional Soxhlet extraction and a more recent solvent extraction development (microwave assisted extraction) for the extraction of PAHs from various soils. After optimization of the operating parameters used in both SFE and microwave extraction, both techniques were found to increase the total concentration of PAHs extracted from a test soil. Experimental design techniques were used to assist the optimization process and were found to improve the validity of the results as well as reduce the number of experiments required when compared to more conventional optimization procedures. However having stated this, no attempt was made to optimize the Soxhlet operating conditions (*i.e.* solvent choice) as this was a standard method employed by the funding laboratory and the work was meant as an investigation into the possibility of replacing this time-consuming way of extracting solid samples with a more rapid alternative. Further work involving different soil matrices indicated that the amount of analyte removed from the soil by both SFE and microwave extraction was dependent on the soil matrix itself and not significantly affected by instrumental operating conditions. However in all cases, the more modern techniques were capable of, at worst, extracting the same amount of PAHs from the soil as Soxhlet extraction, in a fraction of the time and with a significant reduction in solvent consumption.

The conclusion that was drawn from the above work about the effect of the soil matrix on the extraction efficiency of both techniques prompted further investigation into the role that soil matrix plays on recovery of analytes. The test analytes chosen for the study were those already used in the first part of the work involving aqueous matrices and included OCPs, OPPs, and herbicides. The work involved the spiking of previously dried and characterized soils with the various classes of pesticide, prior to supercritical fluid elution using conditions derived from previous studies involving Celite as an inert matrix. In almost all cases (with the exception of herbicides) the soil matrix had a direct effect on the recovery of the pesticides, with organic matter content being found to have a profound effect on the ability of SFE to recover the spiked analytes. No attempt was made to elucidate the operating conditions required to quantitatively remove the test compounds from the various soils or to compare the performance of SFE with other extraction techniques already investigated (Soxhlet and microwave assisted extraction), as the aim of the study was purely to assess the involvement that soil matrix has on extraction efficiency in SFE. Future studies may involve optimizing operating conditions to allow efficient recovery of all analytes and

in particular may benefit from the use of native soils (as used in the PAH study) as these may be a more reliable indicator to true extraction efficiency.

As a general conclusion to the work carried out on soil matrices, microwave extraction as it stands is unlikely to become routine if there still remains no means to monitor the extraction temperature and pressure in each individual extraction vessel since this may vary greatly depending on solid matrix type. This reluctance has been demonstrated by the US EPA, who after several years have still not published a protocol for the extraction of organic analytes from solid samples by microwave technology.

SFE also remains in the background despite being acknowledged by the EPA (for the extraction of total petroleum hydrocarbons) because of the lack of automation and the availability of 'turn-key' methodology. The ease of use, simplicity of operation and the minimal requirement for organic solvents make supercritical fluid extraction a method of choice for environmental matrices. However, in order to achieve accurate and reliable data there is a requirement for experimental data using accredited quality assurance procedures. The evidence so far is that more work is needed to achieve the reliability in data quality expected of a technique with the promise of SFE. This will only occur with the advent of more robust commercial instrumentation capable of handling the types of matrix and extracts routinely determined in laboratories and an increase in the level of automation. Unfortunately, research by commercial companies is governed by sales and instrumental organizations are reluctant to invest in these changes without the necessary turnover of existing instruments. However, this development may be driven by tighter controls on solvent usage and ever increasing disposal cost making extraction techniques such as Soxhlet which use large volumes of solvent to become prohibitive. Until this occurs, SFE seem set to be used almost exclusively in an academic research environment or in applications were solvents cannot be successfully used.

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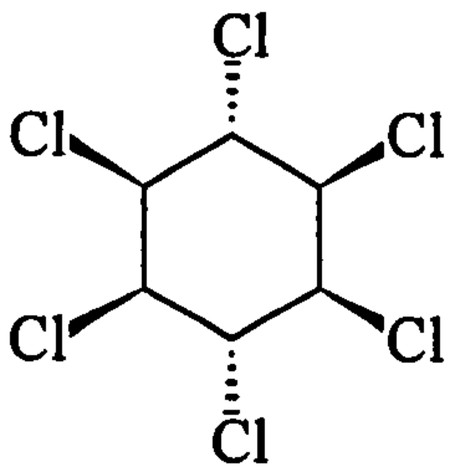
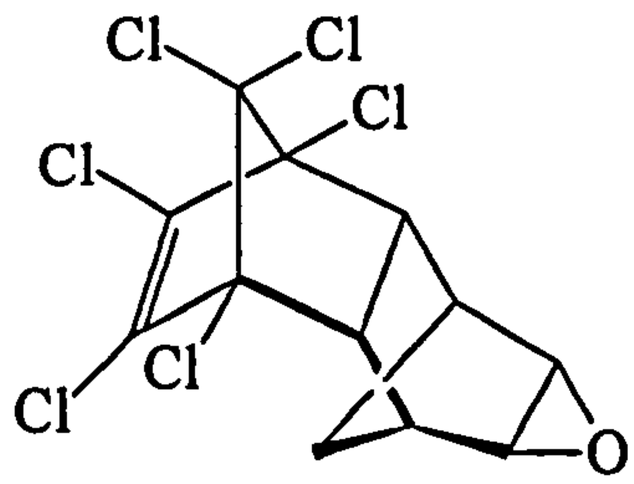
APPENDICES

Appendix 1

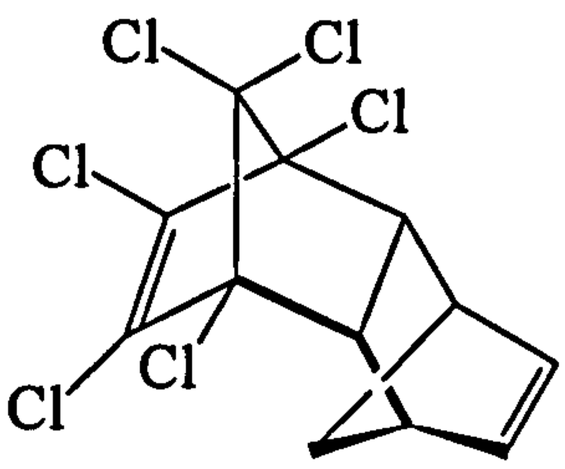
Chemical Structures of the Organochlorine Pesticides



Lindane



Dieldrin



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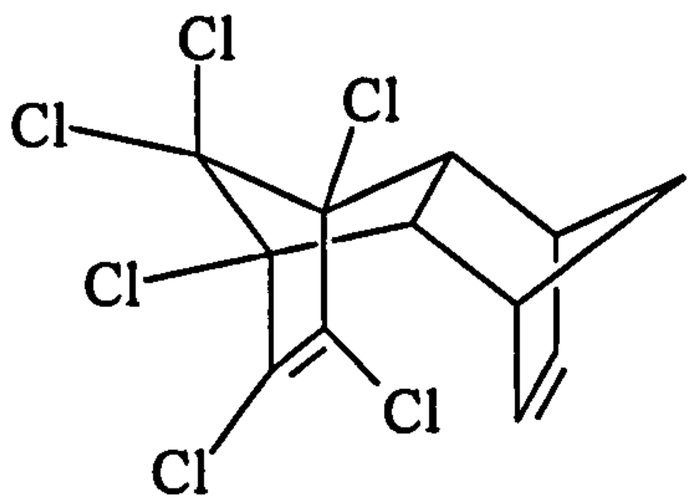
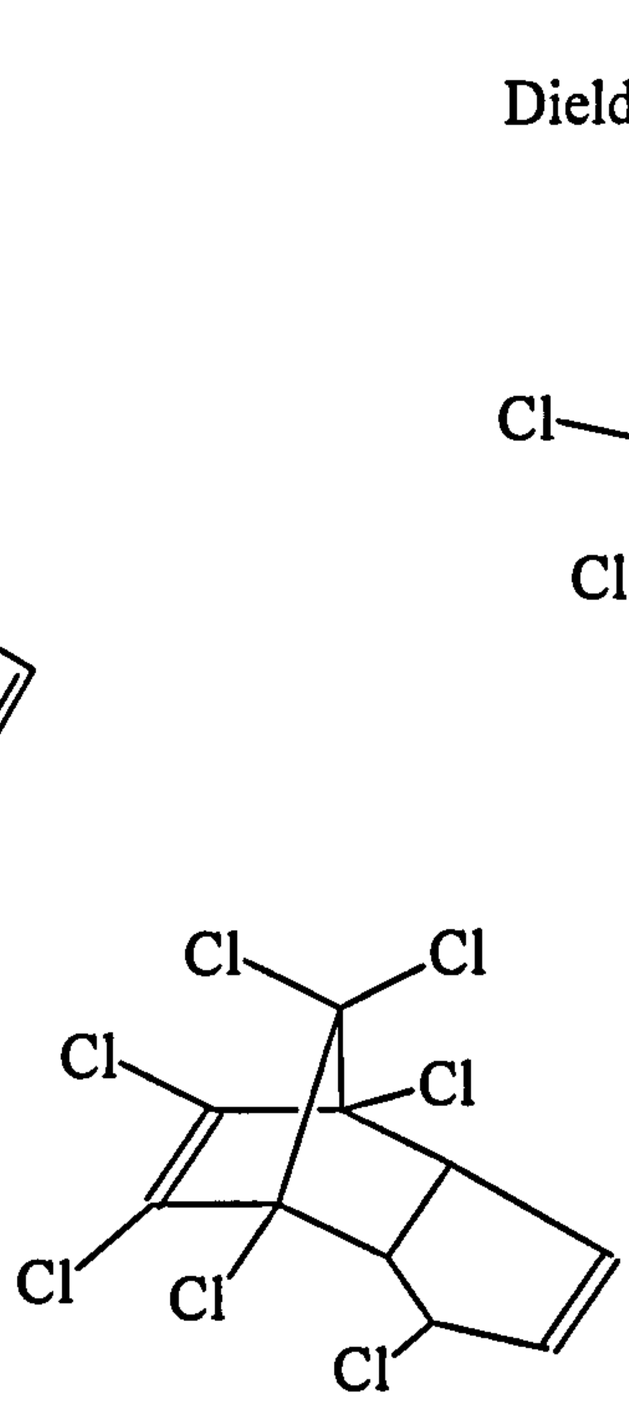
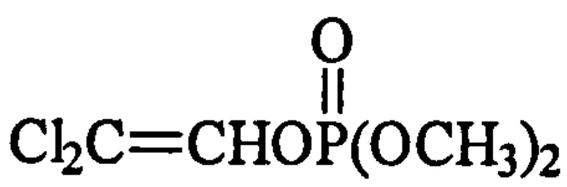


Isodrin

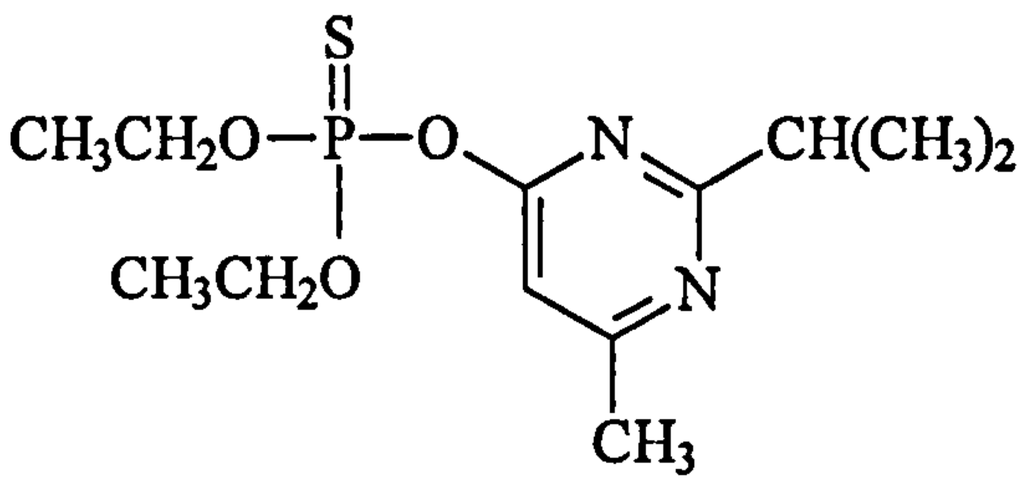


Heptachlor

Chemical Structures of the Organophosphorus Pesticides



Dichlorvos



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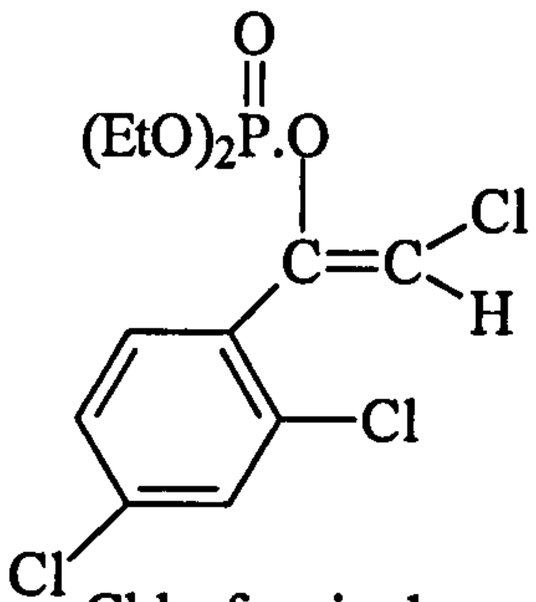
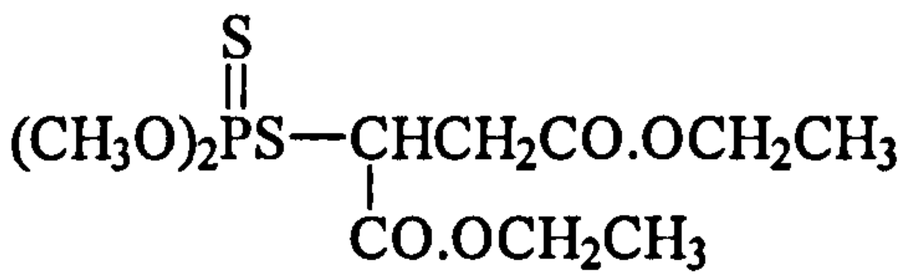
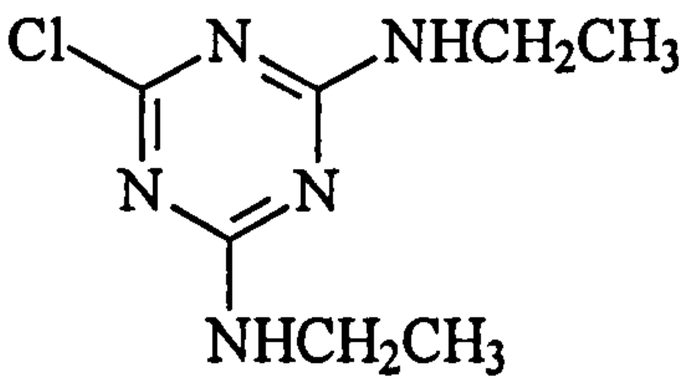


Chlorfenvinphos

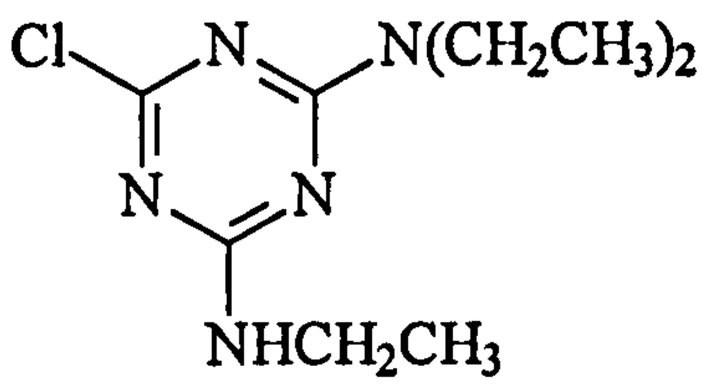


Malathion

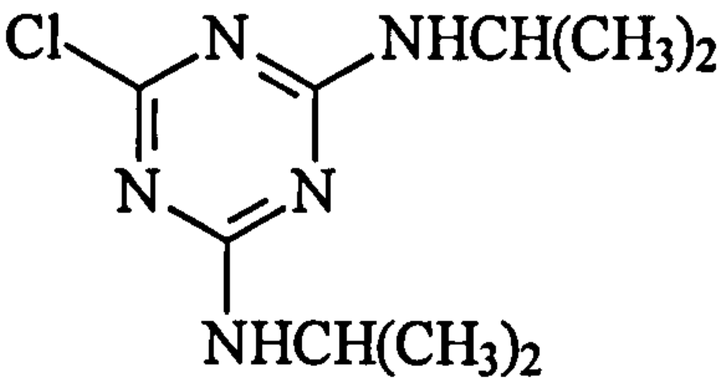
Chemical Structure of both the s-Triazine and Urea Herbicides



Simazine



Trietazine



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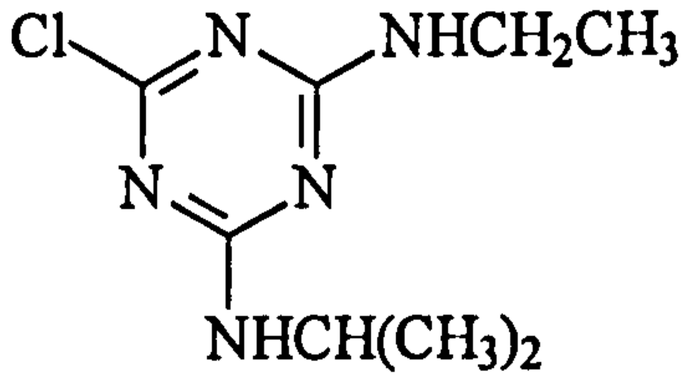
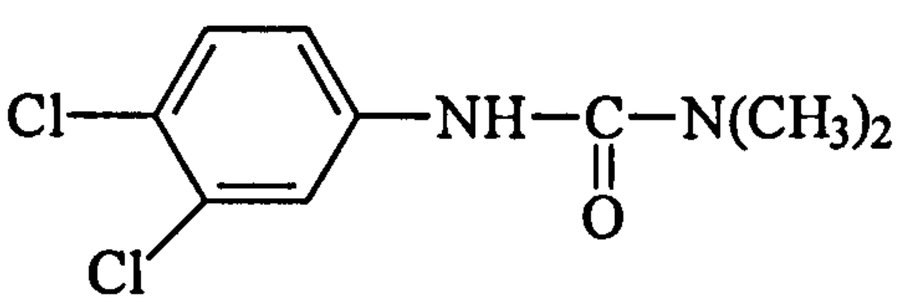
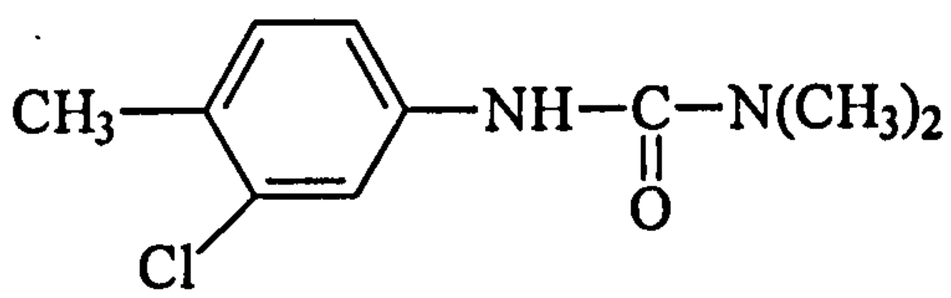


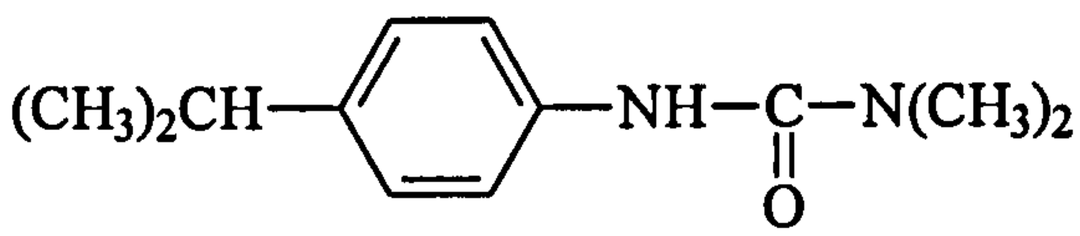
Atrazine



Diuron

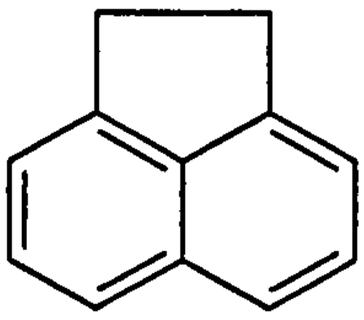


Chlortoluron



Isoproturon

Chemical Structure of the Polycyclic Aromatic Hydrocarbons



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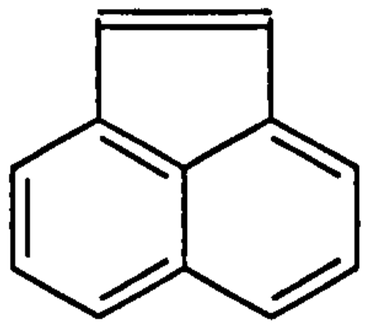

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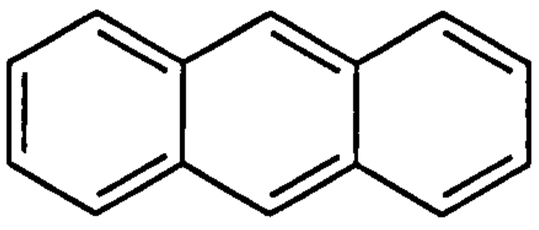
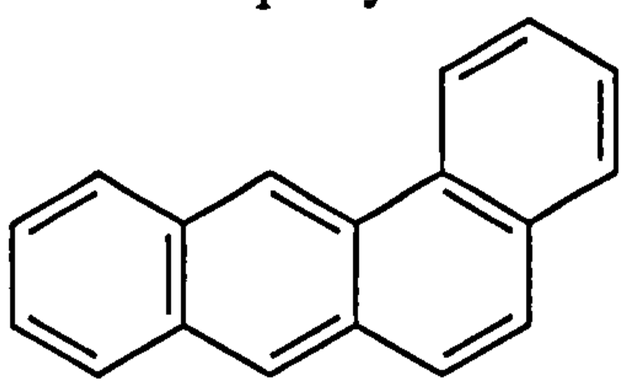
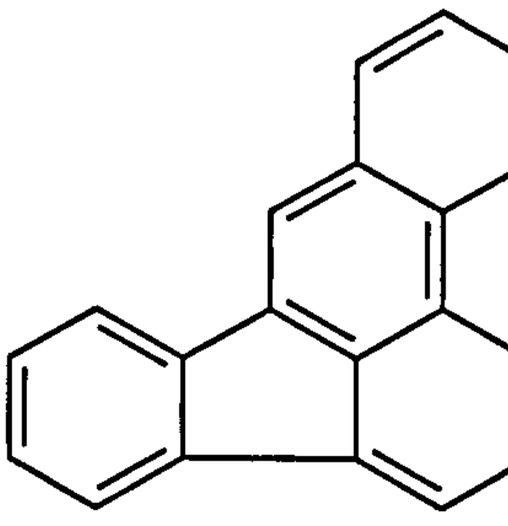


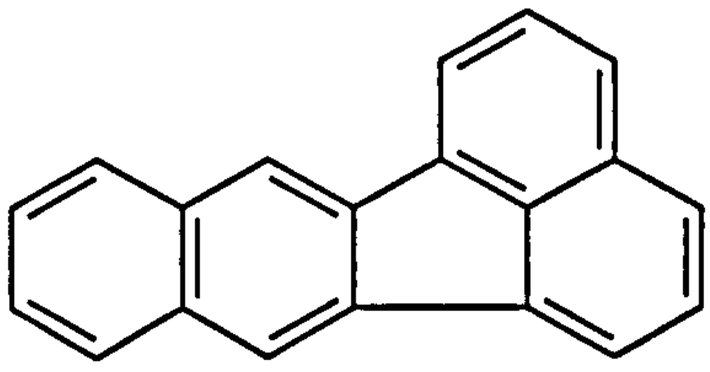
Anthracene



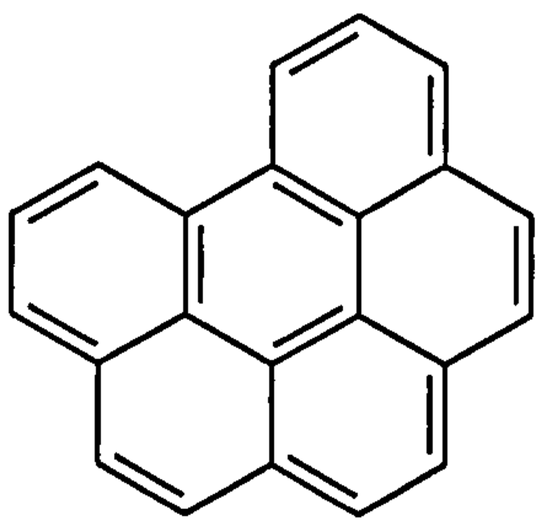
Benz(a)anthracene



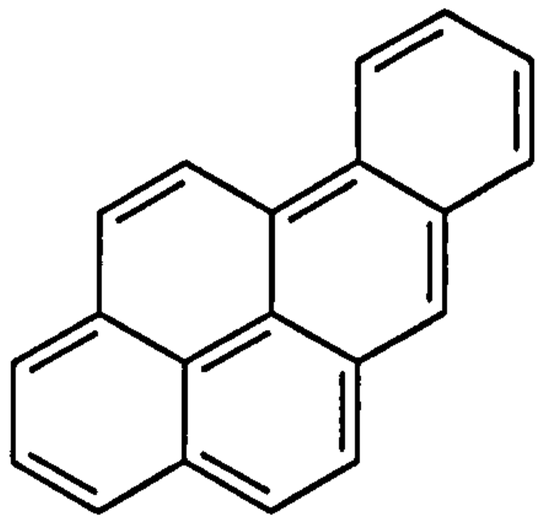
Benzo(b)fluoranthene



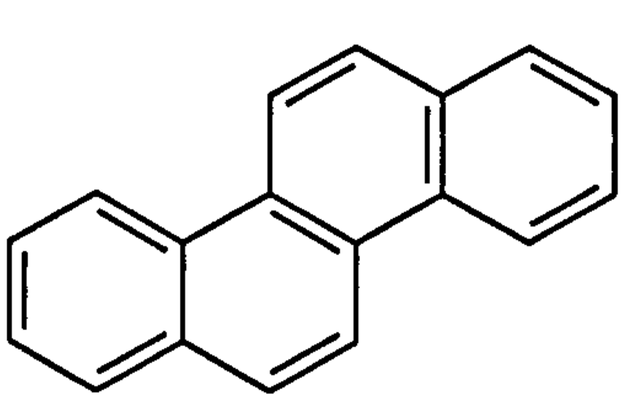
Benzo(k)fluoranthene



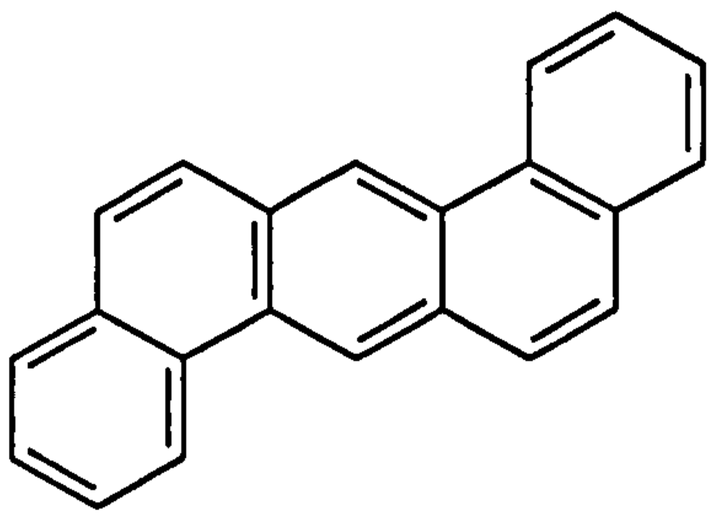
Benzo(ghi)perylene



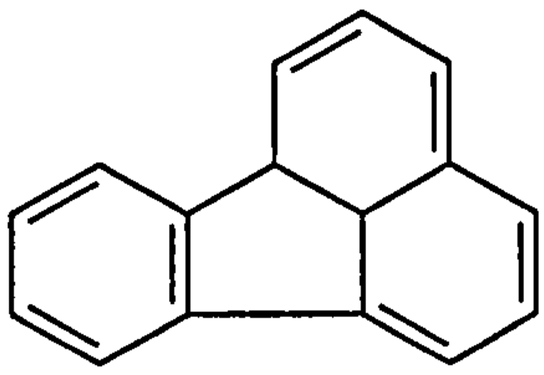
Benzo(a)pyrene



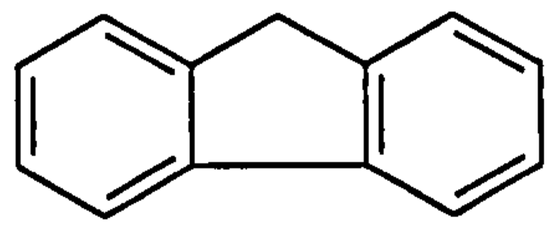
Chrysene



Dibenz(a,h)anthracene



Fluoranthene



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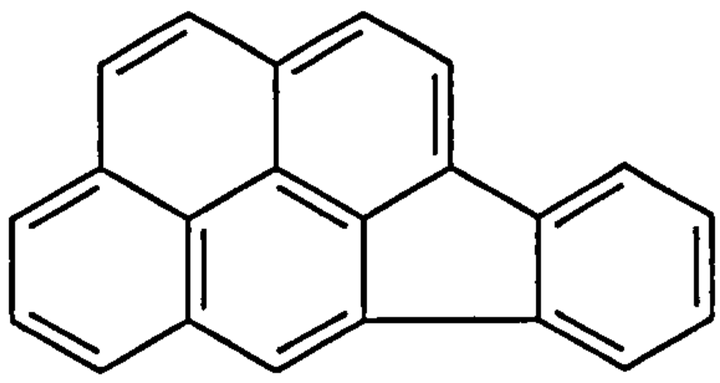
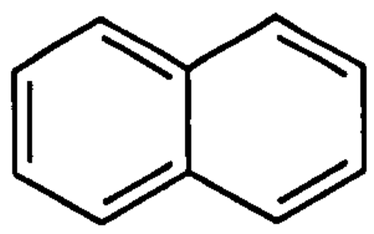
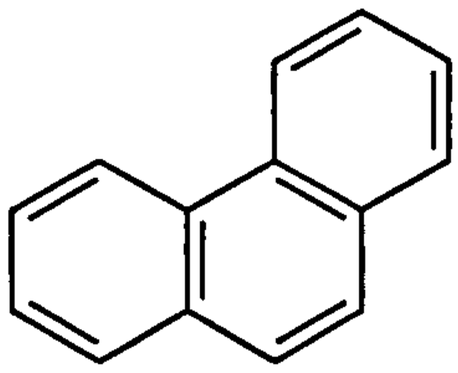


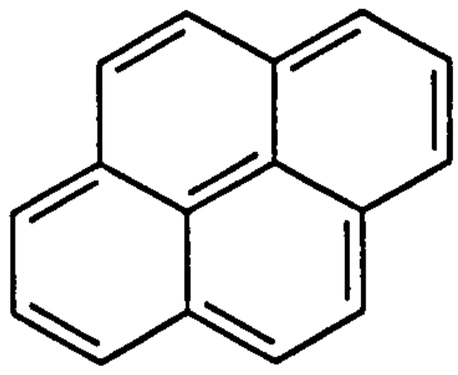
Indeno(1,2,3-cd)pyrene



Naphthalene

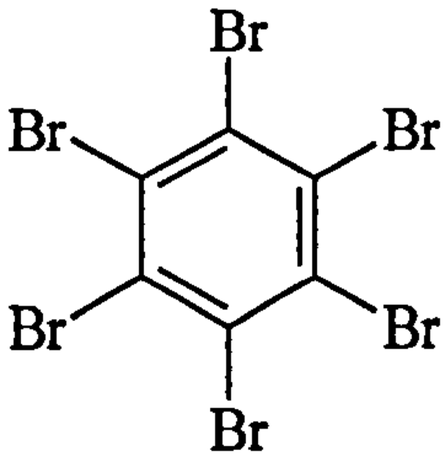


Phenanthrene



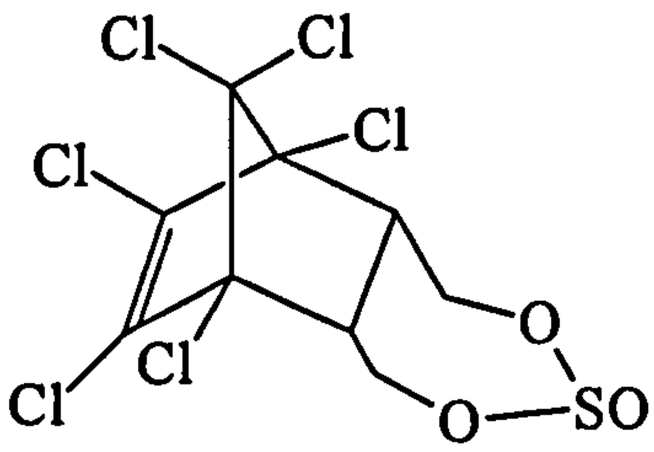
Pyrene

Chemical Structures of the Internal Standards

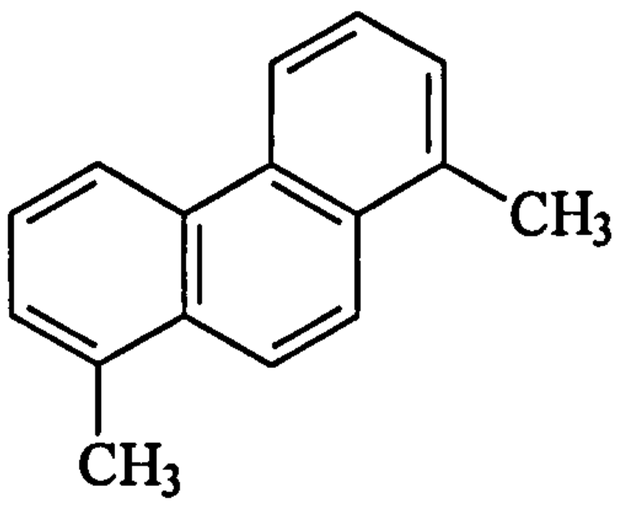


Hexabromobenzene

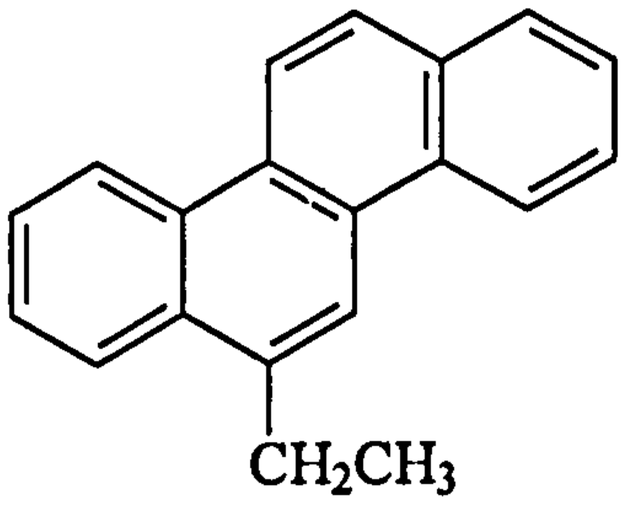
Demeton-s-methyl



β–Endosulphan



3,6-Dimethylphenanthrene



6-Ethylchrysene

Appendix 2

Example Calculation of the Main and Interaction Effects for Pressure and Temperature in a 2^2 Factorial Design.

Example: Main Effect for Lindane

Two responses at high pressure (y_2 and y_4)
The average response at high pressure, y^+ is;

$$y^+ = \tfrac{1}{2} [y_2 + y_4]$$

similarly at low pressure;

$$y^- = \tfrac{1}{2} [y_1 + y_3]$$

Therefore the main effect of pressure, Ep is calculated by;

$$Ep = \tfrac{1}{2} [\tfrac{1}{2} (y_2 + y_4) - \tfrac{1}{2} (y_1 + y_3)]$$

$$Ep = \tfrac{1}{4} [-y_1 + y_2 - y_3 + y_4]$$

also for temperature, Et

$$Et = \tfrac{1}{4} [-y_1 - y_2 + y_3 + y_4]$$

Thus, for lindane;

$$Ep = \tfrac{1}{4} [-49.9 + 85.3 - 41.1 + 84.1] = 19.6\ \%$$

and

$$Et = \tfrac{1}{4} [-49.9 - 85.3 + 41.1 + 84.1] = -2.5\ \%$$

Example: Interaction Effects for Lindane

The effect of temperature at low pressure level is;

$$Et(p^-) = \tfrac{1}{2} [y_1 - y_3]$$

while at the high pressure level;

$$Et(p^+) = \tfrac{1}{2} [y_2 - y_4]$$

for lindane;

$$Et(p^-) = 4.4$$
$$Et(p^+) = 0.6$$

Therefore the overall interaction Etp, is calculated by;

$$Etp = \tfrac{1}{2} [Et(p^-) - Et(p^+)] = 1.9\ \%$$

Appendix 3

Recoveries of Organochlorine Pesticides Directly Extracted by SFE from an Aqueous Matrix

Extraction Time (min)		Flow-Rate (ml min^{-1})		
	0.7	**1.0**	**1.0 (+ salt)**	**1.5**
15	24.3	17.3	26.6	15.2
30	35.0	36.2	34.4	34.8
60	43.8	47.6	55.4	49.4
90	43.2	58.9	64.5	49.6
120	47.3	72.5	66.6	54.6

Table A3.1 Extraction Recoveries (%) for Lindane at Different Flow-Rates and with the Addition of Salt.

Extraction Time (min)		Flow-Rate (ml min^{-1})		
	0.7	**1.0**	**1.0 (+ salt)**	**1.5**
15	20.5	12.0	22.0	13.1
30	28.3	18.0	26.9	22.3
60	33.9	28.7	44.7	38.9
90	38.8	36.1	50.6	38.5
120	45.2	49.9	48.8	56.7

Table A3.2 Extraction Recoveries (%) for Aldrin at Different Flow-Rates and with the Addition of Salt.

Extraction Time (min)		Flow-Rate (ml min^{-1})		
	0.7	**1.0**	**1.0 (+ salt)**	**1.5**
15	16.5	18.0	25.8	27.3
30	27.0	36.1	37.3	42.5
60	29.4	54.7	63.1	53.5
90	35.6	49.2	49.0	59.2
120	42.1	70.0	55.2	56.7

Table A3.3 Extraction Recoveries (%) for Dieldrin at Different Flow-Rates and with the Addition of Salt.

Extraction No.	Lindane	Aldrin (% Recovery)	Dieldrin
1	21.6	19.1	27.5
2	18.1	15.9	23.1
3	19.4	16.3	22.5
4	20.4	17.5	24.1
5	19.2	16.9	25.6
Average	19.7	17.1	24.6
Standard Deviation	1.3	1.3	2.0
% RSD	6.7	7.3	8.2

Table A3.4 Direct Extraction from Water Repeatability Study.

Recoveries of Organochlorine Pesticides Extracted from an Aqueous Matrix Using a SFE-SPE Approach

Extraction Number	Lindane	% Recovery (10 µg) Aldrin	Dieldrin
1	65.3	103.0	80.0
2	78.8	89.9	96.6
3	88.5	96.8	99.6
4	75.5	104.4	90.2
Average	77.0	98.5	91.6
Standard Deviation	9.6	6.6	8.7
% RSD	12.4	6.7	9.5

Table A3.5 Combined Solid-Phase Extraction - Supercritical Fluid Extraction of Organochlorine Pesticides from an Aqueous Sample.

Results of Solid-Phase Microextraction Study Involving s-Triazine Herbicides

Initial Column Temp. (°C)		Peak Area / (Height)		
	Simazine	Atrazine	Propazine	Trietazine
40	644 (199)	1696 (526)	4574 (1434)	38207 (12155)
50	930 (248)	2191 (627)	5867 (1611)	44433 (12989)
60	589 (204)	1809 (564)	4858 (1567)	41648 (13066)
70	551 (192)	1713 (534)	4835 (1495)	40253 (12769)
80	899 (238)	2039 (628)	5201 (1536)	43509 (13306)
90	1511 (293)	2097 (580)	6177 (1615)	45061 (13724)
100	752 (226)	1721 (530)	4738 (1380)	41433 (12334)

Table A3.6 Effect of Initial Column Focusing Temperature on Peak Area (and Height) using the 7 μm Fibre.

Desorption Temperature (°C)		Peak Area		
	Simazine	Atrazine	Propazine	Trietazine
320	657	1891	4809	47270
310	756	1964	5424	52369
300	780	2061	5802	52845
290	774	2059	5286	49969
280	791	2021	5222	50178
270	761	2048	5092	49856
260	789	1943	5209	50457
250	772	1951	5368	49527
240	774	2219	5186	52464
230	737	1885	5038	49343
220	770	1968	5573	56865

Table A3.7 Effect of Desorption Temperature on Peak Area for 7 μm Fibre.

Desorption Temperature (°C)		Peak Area		
	Simazine	Atrazine	Propazine	Trietazine
220 Blank Desorb	28594 -	69161 -	138979 -	379537 -
210 Blank Desorb	23033 -	58300 -	114863 -	326020 -
200 Blank Desorb	23822 -	60188 -	122038 -	347459 -
190 Blank Desorb	21932 -	53911 -	107127 -	309529 2639
180 Blank Desorb	23850 -	60710 -	128298 571	428847 1247
170 Blank Desorb	24435 -	60412 -	126019 580	395507 1153
160 Blank Desorb	23277 -	58668 -	116580 741	315841 1287
150 Blank Desorb	22241 850	55882 984	111684 1212	299396 1688
140 Blank Desorb	18954 1434	50391 2454	104452 4180	318654 8115
130 Blank Desorb	18057 5391	47988 9489	101277 15245	293166 30369
120 Blank Desorb	10638 9539	31816 20398	66127 34688	189662 66777

Table A3.8 Effect of Desorption Temperature on Peak Area for 100 μm Fibre.

Adsorption Time (min)		Peak Area		
	Simazine	Atrazine	Propazine	Trictazine
0.5	2382	4149	5928	13205
1.0	3943	7119	10565	27469
2.0	4950	10655	18400	55558
5.0	8548	20135	41601	170989
7.5	10292	25377	56036	285181
10.0	11260	28753	66882	394713
15.0	11785	30942	77381	617468

Table A3.9 Effect of Adsorption Time on Peak Area using a 100 μm Fibre.

Repeat Number	Desorption Time (min)		Peak Area		
		Simazine	Atrazine	Propazine	Trietazine
1	15	29803	69277	127256	299896
2	15	24363	55625	102897	253158
3	15	27851	58833	110257	272721
1 Blank	5 Desorption	22486 -	55416 -	104009 399	258902 251
2 Blank	5 Desorption	24027 -	55933 -	105579 631	257619 -
3 Blank	5 Desorption	27014 -	59001 -	109567 409	271129 321

Table A3.10 Effect of Desorption Time on Peak Area for Multiple Extractions.

Repeat Number		Peak Area		
	Simazine	Atrazine	Propazine	Trietazine
1	406	1093	2549	4563
2	471	911	2709	5254
3	428	733	2402	6368
Average	435	912	2553	5395
Standard Deviation	33.1	180.0	153.0	910.7
% RSD	7.6	19.7	6.0	16.9

Table A3.11 Repeatability Study on Multiple Extractions of a Low Concentration Solution.

Appendix 4

Pesticide	Log P (Shake flask method)
Lindane	3.72
Aldrin	6.50
Dieldrin	4.32
Dichlorvos	1.47
Diazinon	3.14
Malathion	2.84

Table A4.1 Octanol-Water Partition Coefficients (Log P) for Organochlorine and Organophosphorus Pesticides.[47]

Results of Selectivity Study Involving Organochlorine and Organophosphorus Pesticides

Compound	Extraction Pressure (MPa)								
	7.5	10.5	13.5	17.5	20.5	25.0	30.0	35.0	40.0
Dichlorvos	0	9.3	11.4	22.4	27.2	45.4	49.1	49.8	59.2
Diazinon	0.5	2.0	2.6	7.4	5.9	5.9	10.6	21.9	25.5
Malathion	1.3	3.8	7.6	30.6	39.1	44.9	60.0	63.4	76.2
Lindane	3.9	72.8	98.6	97.8	101.8	89.4	104.8	97.6	101.9
Aldrin	2.0	89.6	88.4	107.6	103.8	107.5	99.5	90.2	115.8
Dieldrin	2.4	47.0	66.1	73.4	94.1	93.2	104.6	101.8	107.9

Table A4.2 Percentage Recoveries of OCPs and OPPs as a Function of Extraction Pressure (Carbon Dioxide Only).

Compound				Extraction pressure (MPa)					
	7.5	**10.5**	**13.5**	**17.5**	**20.5**	**25.0**	**30.0**	**35.0**	**40.0**
Dichlorvos	11.9	59.5	52.3	49.3	49.0	42.2	37.8	41.5	34.5
	(11.9)	**(68.8)**	**(63.7)**	**(71.7)**	**(76.2)**	**(87.6)**	**(86.9)**	**(91.3)**	**(93.7)**
Diazinon	1.4	50.1	56.3	65.9	75.0	73.9	80.5	71.7	66.2
	(1.9)	**(52.1)**	**(58.9)**	**(73.3)**	**(80.9)**	**(79.8)**	**(91.1)**	**(93.6)**	**(91.7)**
Malathion	1.0	74.5	70.3	41.9	40.2	36.2	19.1	34.0	28.5
	(2.3)	**(78.3)**	**(77.9)**	**(72.5)**	**(79.3)**	**(81.1)**	**(79.1)**	**(97.4)**	**(104.7)**
Lindane	3.4	11.8	8.5	2.4	N.D	N.D	N.D	N.D	N.D
	(7.3)	**(84.6)**	**(107.1)**	**(100.2)**	**(101.8)**	**(89.4)**	**(104.8)**	**(97.6)**	**(101.9)**
Aldrin	5.3	17.2	9.4	3.9	N.D	N.D	N.D	N.D	N.D
	(7.3)	**(106.8)**	**(97.8)**	**(111.5)**	**(103.8)**	**(107.5)**	**(99.5)**	**(90.2)**	**(115.8)**
Dieldrin	2.1	35.7	26.8	12.3	N.D	N.D	N.D	N.D	N.D
	(4.5)	**(82.7)**	**(92.9)**	**(85.7)**	**(94.1)**	**(93.2)**	**(104.6)**	**(101.8)**	**(107.9)**

Table A4.3 Percentage Recoveries of OCPs and OPPs as a Function of Extraction Pressure (Carbon Dioxide + 400 µl Methanol).
Total recoveries (first CO_2 extraction and second CO_2 + methanol extraction combined) shown in bold.

* N.D is Not Detected

Results of Selectivity Study Involving Organochlorine Pesticides and both s-Triazine and Urea Herbicides

Compound	Extraction 1 (%)	Extraction 2 (%)	Average (%)
Chlortoluron	109	106	108
Isoproturon	128	123	126
Diuron	103	104	104

Table A4.4 Percentage Extraction Recoveries Obtained from Initial SPE Disk Study with Elution using Methanol.

Compound	Extraction 1 (%)	Extraction 2 (%)	Total (%)
Simazine	N.D	N.D	0
Propazine	3	2	5
Trietazine	2	N.D	2
Chlortoluron	2	2	4
Isoproturon	2	2	4
Diuron	2	1	3

Table A4.5 Percentage Recoveries of Herbicides using the Carlo Erba SFE at 13.5 MPa and 50 °C, using pure CO_2.

* ND is Not Detected

Compound	Extraction 1 (%)	Extraction 2 (%)	Total (%)
Simazine	85.9	17.7	104.0
Propazine	84.7	11.2	95.9
Trietazine	84.1	10.9	95.0
Chlortoluron	69.4	22.3	91.7
Isoproturon	66.1	20.9	87.0
Diuron	59.7	24.4	84.1

Table A4.6 Percentage Recoveries of Herbicides using the Carlo Erba SFE at 40 MPa and 50 °C with the Addition of 400 µl of Methanol Directly to the SPE Disk.

Compound	Mean Percentage Recovery	Individual Recovery	Standard Deviation	% RSD
Heptachlor	91.7	93.5, 92.9, 91.7, 92.3, 88.2	2.1	2.3
Isodrin	101.6	104.9, 114.5, 98.1, 96.3, 94.1	8.3	8.1
Dieldrin	84.8	83.7, 88.2, 85.1, 85.4, 81.7	2.4	2.8
Simazine	1.6	3.8, 1.5, 0.9, 1.0, 0.9	1.2	76.8
Propazine	2.7	10.7, 2.0, 0.4, N/D, 0.4	4.5	168.0
Trietazine	3.5	9.2, 2.9, N/D, 2.2, 3.1	3.4	98.4
Chlortoluron	4.4	9.0, 5.4, 1.5, 1.7, 4.5	3.1	69.6
Isoproturon	3.9	7.3, 3.9, 2.3, 2.0, 4.0	2.1	54.0
Diuron	4.3	8.8, 4.3, 2.7, 2.8, 2.7	2.6	61.7

Table A4.7 Percentage Recoveries of OCPs and Herbicides using CO_2 only (Jasco SFE).

Compound	Mean Percentage Recovery	Individual Recovery	Standard Deviation	% RSD
Simazine	100.4	82.6, 108.3, 112.3, 90.7, 108.1	13.0	12.9
Propazine	88.5	90.7, 80.3, 93.4, 83.0, 95.3	6.6	7.4
Trietazine	86.6	76.2, 81.8, 92.5, 87.1, 95.2	7.8	9.0
Chlortoluron	86.1	75.0, 82.4, 90.7, 86.2, 96.0	8.0	9.3
Isoproturon	90.2	93.6, 83.4, 92.6, 87.2, 94.3	4.7	5.2
Diuron	87.8	82.1, 80.5, 90.7, 88.4, 97.3	6.8	7.7

Table A4.8 Percentage Recoveries of Herbicides using modified CO_2 (Jasco SFE).

Appendix 5

Compound	Soxhlet 1	Soxhlet 2	Soxhlet 3	Soxhlet 4	Soxhlet 5	Soxhlet 6	Average	% RSD
Naphthalene	7.5	4.6	5.8	5.6	5.5	6.9	6.0	17.5
Acenaphthylene	1.6	0.5	0.5	0.5	0.5	0.3	0.7	73.2
Acenaphthene	1.0	1.8	2.3	2.4	1.9	1.3	1.9	30.1
Fluorene	2.8	2.4	3.3	1.6	2.7	2.0	2.5	24.3
Phenanthrene	1.5	0.8	1.7	0.8	0.9	0.5	1.0	45.8
Anthracene	2.9	1.8	2.3	3.5	1.9	1.3	2.3	34.6
Fluoranthene	1.1	0.9	1.3	1.0	1.4	1.0	1.1	17.0
Pyrene	1.0	1.2	1.0	0.5	1.4	0.8	1.0	31.5
Benz(a)anthracene	3.7	3.4	2.2	1.9	4.2	1.9	2.9	35.8
Chrysene	5.3	5.3	3.5	1.8	4.2	3.5	3.9	33.4
Benzo(b)fluoranthene	3.8	2.6	2.5	1.7	3.5	2.7	2.8	26.3
Benzo(k)fluoranthene	4.3	3.4	2.0	1.9	5.8	3.0	3.4	44.1
Benzo(a)pyrene	7.2	3.7	1.5	1.8	6.9	3.6	4.1	59.5
Indeno(1,2,3-cd)pyrene	9.2	4.1	2.7	3.3	13.5	3.2	6.0	73.3
Dibenz(a,h)anthracene	14.7	7.7	5.0	3.3	35.0	11.9	12.9	90.0
Benzo(ghi)perylene	10.0	1.8	3.8	2.5	9.7	5.9	5.6	63.4
Total	77.6	46.0	41.4	34.1	99.0	49.8	58.1	43.1

Table A5.1 Results of Soxhlet Extraction using Dichloromethane on Soil 1. (concentration in mg kg^{-1})

Compound	Soxhlet 1	Soxhlet 2	Soxhlet 3	Soxhlet 4	Soxhlet 5	Average	% RSD
Naphthalene	4.9	4.7	4.8	2.7	3.8	4.2	22.4
Acenaphthylene	3.2	2.6	3.0	1.9	2.4	2.6	19.5
Acenaphthene	8.1	6.7	7.0	4.7	5.7	6.4	20.1
Fluorene	10.4	8.8	9.1	6.9	7.6	8.6	15.9
Phenanthrene	62.2	53.7	55.0	46.1	50.2	53.4	11.2
Anthracene	15.4	13.3	13.2	12.4	13.9	13.6	8.2
Fluoranthene	59.6	54.0	52.8	53.5	50.7	54.1	6.1
Pyrene	47.8	42.4	42.4	42.9	39.4	43.0	7.1
Benz(a)anthracene	27.8	23.6	23.3	26.5	25.1	25.3	7.6
Chrysene	28.2	25.7	25.2	26.1	27.7	26.6	4.9
Benzo(b)fluoranthene	19.8	16.0	13.4	12.9	13.4	15.1	19.2
Benzo(k)fluoranthene	13.3	10.8	10.0	10.3	10.6	11.0	12.0
Benzo(a)pyrene	19.3	15.6	15.0	12.6	13.8	15.3	16.6
Indeno(1,2,3-cd)pyrene	10.6	7.1	6.9	5.5	6.1	7.2	27.4
Dibenz(a,h)anthracene	6.8	3.1	3.1	1.8	2.2	3.4	58.3
Benzo(ghi)perylene	10.2	7.6	7.9	5.4	6.9	7.6	23.0
Total	347.6	295.7	291.1	272.2	279.5	297.4	10.0

Table A5.2 Results of Soxhlet Extraction using Dichloromethane on Soil 2. (concentration in mg kg^{-1})

Compound	Soxhlet 1	Soxhlet 2	Soxhlet 3	Average	% RSD
Naphthalene	12.3	11.5	12.6	12.1	4.7
Acenaphthylene	1.9	1.8	1.7	1.8	5.6
Acenaphthene	1.0	0.9	0.9	0.9	6.2
Fluorene	0.8	0.8	0.8	0.8	0.0
Phenanthrene	66.2	70.6	67.0	67.9	3.5
Anthracene	2.4	1.1	2.1	1.9	36.5
Fluoranthene	59.7	56.6	54.0	56.8	5.0
Pyrene	34.8	33.9	34.1	34.3	1.4
Benz(a)anthracene	10.9	10.8	10.9	10.9	0.5
Chrysene	16.2	15.3	15.1	15.5	3.8
Benzo(b)fluoranthene	13.0	13.3	14.0	13.4	3.8
Benzo(k)fluoranthene	7.1	11.2	10.5	9.6	22.8
Benzo(a)pyrene	2.1	2.2	2.0	2.1	4.8
Indeno(1,2,3-cd)pyrene	3.0	2.8	2.7	2.8	5.4
Dibenz(a,h)anthracene	1.9	1.8	1.8	1.8	3.1
Benzo(ghi)perylene	10.3	10.0	9.6	10.0	3.5
Total	243.6	244.6	239.8	242.7	1.0

Table A5.3 Results of Soxhlet Extraction using Dichloromethane on CONTEST Soil (soil 3).
(Concentration in mg kg^{-1})

Run Order	Block	Temperature (°C)	Extraction Time (min)	Solvent Volume (ml)
1	1	105	17.1	46
2	1	80	12.5	40
3	1	80	12.5	40
4	1	105	7.9	34
5	1	56	7.9	46
6	1	56	17.1	34
7	2	105	17.1	34
8	2	105	7.9	46
9	2	56	7.9	34
10	2	56	17.1	46
11	2	80	12.5	40
12	2	80	12.5	40
13	3	80	12.5	30
14	3	80	12.5	40
15	3	120	12.5	40
16	3	80	12.5	50
17	3	80	20.0	40
18	3	80	12.5	40
19	3	80	5.0	40
20	3	40	12.5	40

Table A5.4 The Calculated Levels (Design Expert) for the Central Composite Design used in the Microwave Assisted Extraction Study.

Compound	Micro 1	Micro 2	Micro 3	Micro 4	Average	% RSD
Naphthalene	10.4	9.9	9.7	10.6	10.1	4.1
Acenaphthylene	0.9	0.7	1.4	2.0	1.3	47.3
Acenaphthene	2.8	2.3	4.0	3.3	3.1	23.2
Fluorene	5.5	4.6	3.7	3.7	4.4	19.5
Phenanthrene	1.1	1.6	1.1	1.2	1.2	21.7
Anthracene	3.6	1.2	3.0	1.7	2.4	46.7
Fluoranthene	0.8	0.8	1.1	2.1	1.2	51.9
Pyrene	1.7	0.6	1.2	1.9	1.4	40.9
Benz(a)anthracene	4.2	3.5	2.5	4.3	3.6	22.0
Chrysene	2.3	1.7	4.7	3.1	2.9	44.5
Benzo(b)fluoranthene	1.2	4.1	3.1	6.7	3.8	60.4
Benzo(k)fluoranthene	1.8	6.7	4.2	3.0	3.9	53.1
Benzo(a)pyrene	4.1	5.9	2.6	9.6	5.8	47.1
Indeno(1,2,3-cd)pyrene	3.9	9.4	3.5	12.7	7.4	60.9
Dibenz(a,h)anthracene	8.5	6.6	10.5	8.2	8.7	19.6
Benzo(ghi)perylene	8.6	9.5	3.7	6.7	7.1	36.0
Total	61.4	69.1	60.0	80.8	68.3	13.2

Table A5.5 Results of Microwave Extraction using Dichloromethane on Soil 1. (concentration in mg kg^{-1})

Compound	Micro 1	Micro 2	Micro 3	Micro 4	Average	% RSD
Naphthalene	5.9	5.4	6.9	5.5	5.9	11.6
Acenaphthylene	3.2	3.1	2.9	2.7	3.0	7.5
Acenaphthene	8.0	7.1	8.3	6.8	7.6	9.5
Fluorene	9.7	8.3	9.7	7.9	8.9	10.5
Phenanthrene	57.3	51.0	64.4	49.2	55.5	12.4
Anthracene	17.3	16.1	17.1	11.3	15.5	18.2
Fluoranthene	51.7	45.3	56.9	41.4	48.8	14.0
Pyrene	40.1	34.9	44.6	32.2	38.0	14.5
Benz(a)anthracene	22.1	19.1	23.3	18.0	20.6	12.1
Chrysene	25.2	21.0	26.7	18.6	22.9	16.3
Benzo(b)fluoranthene	16.6	12.1	17.6	11.3	14.4	22.0
Benzo(k)fluoranthene	8.7	8.5	9.6	9.1	9.0	5.4
Benzo(a)pyrene	13.9	12.1	14.1	11.2	12.8	11.0
Indeno(1,2,3-cd)pyrene	7.7	5.9	6.8	5.7	6.5	14.1
Dibenz(a,h)anthracene	4.1	3.1	3.4	2.5	3.3	20.3
Benzo(ghi)perylene	7.5	7.6	7.6	6.3	7.3	8.8
Total	299.0	260.6	319.9	239.7	279.8	13.0

Table A5.6 Results of Microwave Extraction using Dichloromethane on Soil 2. (concentration in mg kg^{-1})

Compound	80/20*	70/30	60/40	50/50	40/60	30/70	20/80	10/90	0/100
Naphthalene	6.7	8.5	9.1	8.4	9.5	11.2	11.2	13.7	13.5
Acenaphthylene	3.4	4.5	3.5	3.5	4.7	4.4	4.2	5.2	5.0
Acenaphthene	8.9	10.4	10.0	9.9	11.3	14.2	14.2	16.6	16.7
Fluorene	10.6	12.5	10.1	10.8	15.6	15.0	15.0	17.6	17.5
Phenanthrene	61.2	54.3	56.7	57.4	57.6	72.7	73.7	85.5	86.6
Anthracene	15.7	17.6	18.6	18.3	20.7	22.9	22.8	26.4	26.4
Fluoranthene	51.5	61.1	59.6	58.8	61.0	75.2	74.8	87.6	89.2
Pyrene	39.5	49.3	49.3	48.1	51.2	61.5	61.0	69.2	70.2
Benz(a)anthracene	21.9	21.0	21.4	20.2	20.6	26.6	26.4	24.6	25.3
Chrysene	25.0	21.0	19.9	19.0	20.2	26.9	26.5	24.8	25.4
Benzo(b)fluoranthene	12.1	17.2	18.3	17.4	19.3	14.5	15.0	18.9	13.7
Benzo(k)fluoranthene	9.6	13.7	12.8	13.2	13.8	15.4	15.6	8.8	10.4
Benzo(a)pyrene	12.5	19.9	19.5	18.6	20.0	26.0	26.0	24.1	24.6
Indeno(1,2,3-cd)pyrene	5.8	9.3	8.7	8.6	9.5	17.0	17.0	15.8	15.7
Dibenz(a,h)anthracene	2.5	3.5	3.2	3.0	3.7	4.2	4.2	3.8	3.8
Benzo(ghi)perylene	6.9	9.4	8.5	8.1	9.1	14.7	14.5	13.6	13.9
Total	293.4	332.6	328.9	323.1	347.4	421.9	421.7	455.7	457.6

Table A5.7 Results of Microwave Extraction using Various Compositions of Acetone/Hexane to Extract Soil 2 (all extractions performed in duplicate).

(Concentration in mg kg^{-1})

*Composition range from 80 : 20 hexane : acetone to 100 % acetone.

Compound	Repeat 1	Repeat 2	Repeat 3	Repeat 4	Repeat 5	Repeat 6	Average	% RSD
Naphthalene	11.2	12.5	12.2	11.1	12.4	12.4	12.0	5.4
Acenaphthylene	3.7	3.7	3.7	3.7	3.7	3.7	3.7	0.0
Acenaphthene	13.7	15.0	14.7	14.8	13.7	14.9	14.5	4.2
Fluorene	12.4	13.7	13.5	13.6	13.7	13.7	13.4	3.8
Phenanthrene	73.2	77.3	77.1	74.2	72.1	74.7	74.8	2.8
Anthracene	23.6	25.0	24.5	23.5	23.6	24.9	24.2	2.9
Fluoranthene	73.2	76.1	74.5	72.9	70.8	74.8	73.7	2.5
Pyrene	57.1	58.6	57.5	55.6	54.7	57.2	56.8	2.5
Benz(a)anthracene	26.1	26.2	27.0	26.0	24.9	27.4	26.3	3.3
Chrysene	27.3	27.5	28.2	27.2	26.1	27.3	27.3	2.5
Benzo(b)fluoranthene	19.9	18.7	20.8	19.8	18.6	21.2	19.8	5.3
Benzo(k)fluoranthene	19.9	17.5	18.8	17.3	17.4	18.7	18.3	5.7
Benzo(a)pyrene	23.6	23.7	25.7	23.5	23.6	24.9	24.2	3.8
Indeno(1,2,3-cd)pyrene	14.9	15.0	15.9	14.8	14.9	16.2	15.3	4.0
Dibenz(a,h)anthracene	3.7	3.7	3.7	3.7	3.7	3.7	3.7	0.0
Benzo(ghi)perylene	14.9	15.0	15.9	14.8	13.7	14.9	14.9	4.7
Total	419.4	429.2	433.9	416.5	407.6	430.6	422.9	2.4

Table A5.8 Repeatability of Microwave Extraction using 100 % Acetone (extracted at 120 °C for 20 minutes).
(concentration in mg kg^{-1})

Compound	1 (A)	1 (B)	2 (A)	2 (B)	3 (A)	3 (B)	4 (A)	4 (B)	5 (A)	5 (B)	6 (A)	6 (B)
Naphthalene	9.8	10.0	10.0	11.2	11.2	8.8	9.9	9.9	9.7	9.9	10.0	10.0
Acenaphthylene	3.7	3.8	3.7	3.7	3.7	3.8	3.7	3.7	3.6	3.7	3.8	3.8
Acenaphthene	13.4	13.8	13.7	13.7	14.9	12.5	14.8	13.6	13.4	13.7	13.8	13.8
Fluorene	12.2	12.5	11.2	13.7	13.6	12.5	13.6	12.4	13.4	12.4	13.8	12.5
Phenanthrene	68.3	70.1	68.5	72.1	73.2	67.7	71.7	69.5	69.3	72.0	71.4	69.9
Anthracene	22.0	22.5	22.4	22.4	26.1	23.8	22.2	22.3	23.1	23.6	22.5	22.6
Fluoranthene	67.1	70.1	67.3	72.1	74.4	68.9	71.7	69.5	69.3	73.2	71.4	68.9
Pyrene	52.4	53.9	52.3	56.0	57.1	52.6	55.6	53.3	53.5	55.9	55.1	53.9
Benz(a)anthracene	24.4	23.8	23.7	26.1	26.1	23.8	24.7	24.8	24.3	26.1	26.3	25.1
Chrysene	24.4	25.1	24.9	26.1	27.3	25.1	26.0	24.8	25.5	26.1	26.3	25.1
Benzo(b)fluoranthene	18.3	16.3	19.9	17.4	21.1	18.8	17.3	21.1	18.2	18.6	17.5	18.8
Benzo(k)fluoranthene	9.8	11.3	18.7	10.0	12.4	11.3	18.5	14.8	13.4	18.6	10.0	18.8
Benzo(a)pyrene	20.7	21.3	21.2	23.6	23.6	21.3	22.2	22.3	21.9	22.3	22.5	22.6
Indeno(1,2,3-cd)pyrene	12.2	12.5	12.5	13.7	13.7	12.5	12.4	12.4	12.2	13.7	12.5	12.5
Dibenz(a,h)anthracene	2.4	2.5	2.5	3.7	3.7	2.5	2.5	2.5	2.4	2.5	2.5	2.5
Benzo(ghi)perylene	12.2	12.5	11.2	13.7	12.4	11.3	12.4	12.4	12.2	12.4	12.5	11.3
Total	373.3	394.5	383.7	399.2	414.5	399.8	399.2	389.3	385.4	404.7	391.9	391.1

Table A5.9 Results of Central Composite Design Involving Microwave Extraction.

Compound	7 (A)	7 (B)	8 (A)	8 (B)	9 (A)	9 (B)	10 (A)	10 (B)	11 (A)	11 (B)	12 (A)	12 (B)
Naphthalene	11.2	9.9	11.1	11.1	11.0	8.6	11.2	11.2	9.8	11.1	11.0	8.7
Acenaphthylene	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.6
Acenaphthene	13.7	14.8	13.5	14.8	13.5	13.6	13.7	13.7	13.5	13.6	13.4	13.3
Fluorene	12.4	13.6	12.3	14.8	13.5	13.6	13.7	12.5	12.2	13.6	13.4	12.1
Phenanthrene	75.9	75.3	71.3	76.3	74.8	75.3	74.6	74.9	72.3	71.6	70.9	69.1
Anthracene	22.4	22.2	20.9	22.2	22.1	25.9	22.4	22.5	22.1	24.7	22.0	21.8
Fluoranthene	73.4	72.8	58.9	72.6	69.9	74.0	72.1	69.9	69.8	70.4	67.2	66.7
Pyrene	56.0	55.5	51.6	55.4	52.7	55.5	53.4	53.7	52.7	53.1	51.3	50.9
Benz(a)anthracene	26.1	25.9	24.6	25.8	24.5	25.9	26.1	26.2	24.5	24.7	24.4	24.3
Chrysene	27.4	27.1	25.8	27.1	25.8	27.1	26.1	26.2	25.7	24.7	25.7	24.3
Benzo(b)fluoranthene	16.2	19.7	17.2	18.5	18.4	17.3	18.6	18.7	17.1	18.5	19.6	17.0
Benzo(k)fluoranthene	16.2	13.6	16.0	13.5	14.7	12.3	14.9	16.3	12.2	12.4	14.7	15.8
Benzo(a)pyrene	24.9	24.7	23.4	25.8	23.3	24.7	24.9	23.7	23.3	23.5	23.2	23.0
Indeno(1,2,3-cd)pyrene	16.2	17.3	14.8	17.2	14.7	14.8	16.2	15.0	13.5	14.8	14.7	14.6
Dibenz(a,h)anthracene	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.6
Benzo(ghi)perylene	14.9	16.0	14.8	16.0	13.5	14.8	14.9	13.7	13.5	14.8	14.7	13.3
Total	414.3	415.8	393.6	418.5	399.8	398.5	410.2	405.6	389.6	389.9	393.6	382.1

Table A5.9 Continued.

Compound	13 (A)	13 (B)	14 (B)	15 (A)	15 (B)	16 (A)	16 (B)	17 (A)	17 (B)	18 (A)	18 (B)
Naphthalene	9.9	11.2	12.5	11.2	9.9	12.3	10.0	9.9	10.9	12.5	12.2
Acenaphthylene	3.7	3.7	5.0	3.77	3.7	3.7	3.8	3.7	4.9	3.7	4.9
Acenaphthene	13.6	14.9	15.0	16.1	14.8	16.0	15.0	14.8	14.6	16.2	15.8
Fluorene	12.3	14.9	13.7	14.9	14.8	14.8	13.8	12.3	12.2	15.0	14.6
Phenanthrene	72.8	76.9	81.0	80.7	76.6	82.7	75.0	77.6	76.6	81.0	79.2
Anthracene	22.2	23.6	24.9	26.1	27.2	28.4	22.5	23.4	23.1	24.9	24.4
Fluoranthene	71.6	76.9	77.3	79.4	75.3	80.2	72.5	75.1	75.4	77.3	76.7
Pyrene	54.3	58.3	58.6	60.8	58.1	60.5	55.0	57.9	57.2	58.6	58.5
Benz(a)anthracene	24.7	27.3	27.4	28.6	27.2	28.4	26.3	27.1	26.8	27.4	28.0
Chrysene	25.9	28.5	28.7	29.8	27.2	29.6	26.3	28.3	28.0	58.7	28.0
Benzo(b)fluoranthene	17.3	19.8	22.4	23.6	21.0	25.9	21.3	19.7	18.2	23.7	20.7
Benzo(k)fluoranthene	17.3	18.6	17.4	18.6	19.8	14.8	20.0	18.5	17.0	22.4	18.3
Benzo(a)pyrene	22.2	26.0	26.2	26.1	25.9	25.9	23.8	24.6	25.5	24.9	26.8
Indeno(1,2,3-cd)pyrene	14.8	17.4	16.2	16.1	16.1	17.3	15.0	16.0	15.8	16.2	18.3
Dibenz(a,h)anthracene	3.7	3.7	3.7	3.7	3.7	3.7	3.8	3.7	3.7	3.7	4.9
Benzo(ghi)perylene	13.6	16.1	15.0	14.9	14.8	16.0	15.0	14.8	14.6	15.0	17.0
Total	399.9	437.8	445.0	454.3	436.1	460.2	419.1	427.4	424.5	451.2	448.3

Table A5.9 Continued.
Note:- Experiment 14(A) is not included because of a sampling error.

Compound	19 (A)	19 (B)	20 (A)	20 (B)
Naphthalene	10.9	11.2	9.9	10.0
Acenaphthylene	3.6	3.7	3.7	3.7
Acenaphthene	14.6	14.9	13.6	14.9
Fluorene	13.4	14.9	12.3	14.9
Phenanthrene	72.9	75.6	71.5	74.7
Anthracene	21.9	22.3	22.2	2.4
Fluoranthene	70.4	71.9	70.3	74.7
Pyrene	54.6	54.6	53.0	56.1
Benz(a)anthracene	25.5	26.0	24.7	26.2
Chrysene	26.7	27.3	25.9	27.4
Benzo(b)fluoranthene	20.6	21.1	17.3	17.4
Benzo(k)fluoranthene	15.8	19.8	17.3	17.4
Benzo(a)pyrene	24.3	23.6	23.4	24.9
Indeno(1,2,3-cd)pyrene	15.8	14.9	14.8	14.9
Dibenz(a,h)anthracene	3.6	3.7	3.7	3.7
Benzo(ghi)perylene	14.6	13.6	13..6	13.7
Total	409.2	419.1	397.2	417.0

Table A5.9 Continued.
(Concentration in mg kg^{-1})

		Acetone				DCM	
Compound	**Micro 1**	**Micro 2**	**Average**		**Micro 1**	**Micro 2**	**Average**
Naphthalene	13.2	12.7	13.0		13.9	13.0	13.5
Acenaphthylene	1.9	2.2	2.1		2.8	2.8	2.8
Acenaphthene	1.0	0.9	1.0		1.1	1.0	1.1
Fluorene	0.7	0.7	0.7		0.9	0.8	0.9
Phenanthrene	64.9	67.7	66.3		70.5	69.1	69.8
Anthracene	3.1	3.9	3.5		3.3	3.6	3.5
Fluoranthene	53.6	53.0	53.3		54.3	54.6	54.5
Pyrene	36.6	36.1	36.4		35.7	36.0	35.9
Benz(a)anthracene	11.3	10.7	11.0		11.8	11.0	11.4
Chrysene	15.3	14.8	15.1		15.5	14.8	15.2
Benzo(b)fluoranthene	13.0	12.7	12.9		13.4	11.2	12.3
Benzo(k)fluoranthene	12.2	10.8	11.5		10.6	13.1	11.9
Benzo(a)pyrene	1.9	1.8	1.9		2.0	2.0	2.0
Indeno(1,2,3-cd)pyrene	2.8	2.4	2.6		2.8	1.6	2.2
Dibenz(a,h)anthracene	1.7	1.6	1.7		1.7	1.7	1.7
Benzo(ghi)perylene	9.0	9.1	9.1		9.2	9.2	9.2
Total	242.2	241.1	241.7		249.5	245.5	247.5

Table A5.10 Results of Microwave Extraction using Acetone and DCM on CONTEST Soil (3).
(Concentration in mg kg^{-1})

Run Order	Block	Pressure	Temp	Extraction Time	% MeOH
1	1	+1	-1	+1	+1
2	1	-1	+1	+1	+1
3	1	+1	-1	-1	-1
4	1	+1	+1	+1	-1
5	1	-1	+1	-1	-1
6	1	-1	-1	-1	+1
7	1	0	0	0	0
8	1	+1	+1	-1	+1
9	1	-1	-1	+1	-1
10	1	0	0	0	0
11	2	+1	+1	+1	+1
12	2	-1	-1	-1	-1
13	2	+1	+1	-1	-1
14	2	+1	-1	-1	+1
15	2	-1	-1	+1	+1
16	2	-1	+1	-1	+1
17	2	-1	+1	+1	-1
18	2	0	0	0	0
19	2	+1	-1	+1	-1
20	2	0	0	0	0
21	3	0	0	0	0
22	3	0	-α	0	0
23	3	0	0	-α	0
24	3	0	0	+α	0
25	3	-α	0	0	0
26	3	0	0	0	0
27	3	0	0	0	-α
28	3	0	0	0	+α
29	3	0	+α	0	0
30	3	+α	0	0	0

Table A5.11 Full Coded Central Composite Design used in SFE Optimization Study.

Compound	1	2	3	4	5	6	7	8	9	10	11	12
Naphthalene	10.5	11.8	5.2	0.5	11.6	13.4	11.9	10.9	6.1	10.6	11.0	2.1
Acenaphthylene	2.8	3.3	0.6	0.7	1.2	0.1	3.3	2.8	0.5	3.2	3.2	0.5
Acenaphthene	14.3	14.9	3.7	2.2	5.1	15.4	14.0	14.1	3.3	13.2	17.0	2.6
Fluorene	13.0	14.0	2.1	1.2	4.3	13.3	13.6	13.7	2.0	15.2	16.4	2.4
Phenanthrene	77.8	78.4	12.6	10.9	20.9	76.8	78.3	76.4	12.2	86.1	90.0	14.4
Anthracene	27.5	28.0	12.3	9.9	20.4	30.1	29.4	26.3	12.0	31.0	28.3	4.9
Fluoranthene	75.7	77.7	11.3	20.1	14.4	67.6	74.3	75.9	13.0	82.2	84.7	12.0
Pyrene	54.8	58.0	7.7	16.8	9.1	45.4	54.1	56.2	10.5	60.0	62.0	7.7
Benz(a)anthracene	23.4	25.0	2.9	9.2	2.7	14.5	24.2	24.6	3.8	26.4	28.4	2.9
Chrysene	28.7	29.6	3.0	12.5	4.1	18.0	28.3	28.1	5.0	29.5	30.3	3.1
Benzo(b)fluoranthene	12.1	12.0	2.5	15.5	2.9	4.8	19.7	17.3	2.4	21.1	13.7	1.7
Benzo(k)fluoranthene	21.2	25.8	2.4	12.1	2.7	6.2	16.3	19.7	3.3	20.8	21.2	1.2
Benzo(a)pyrene	11.5	13.5	1.6	10.1	2.3	2.7	12.5	13.3	2.0	13.5	24.0	1.0
Indeno(1,2,3-cd)pyrene	9.9	11.7	0.4	11.3	1.3	0.2	11.7	12.6	0.9	12.5	14.0	0.2
Dibenz(a,h)anthracene	0.6	0.8	N.D	0.8	N.D	N.D	0.8	0.9	N.D	0.9	2.4	0.1
Benzo(ghi)perylene	8.0	9.1	0.4	9.2	1.0	0.6	8.2	9.7	0.7	0.3	10.9	0.1
Total	391.8	413.6	68.7	143.0	104.0	309.1	400.6	402.5	77.7	426.5	457.5	56.9

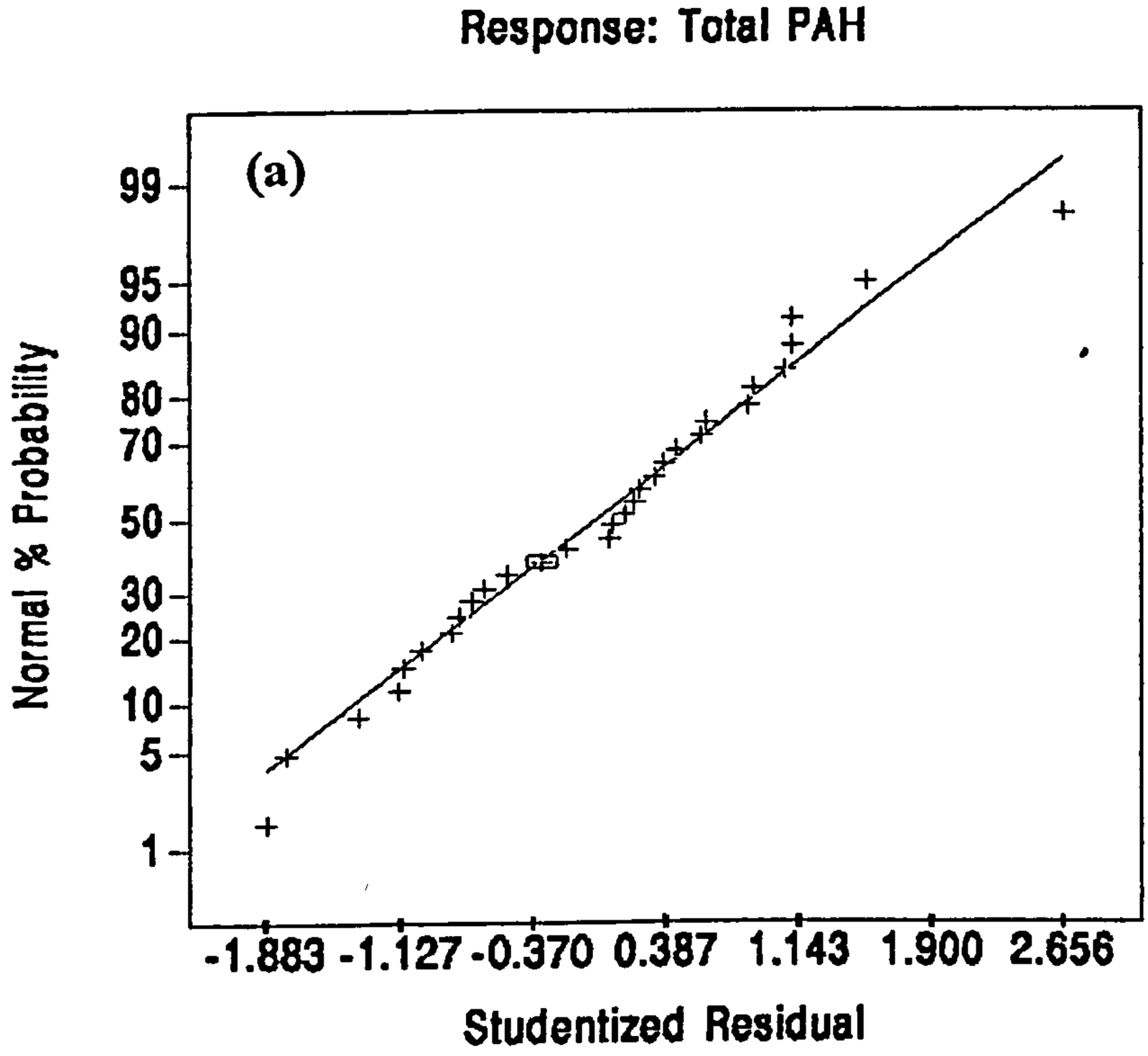
Table A5.12 Results of the SFE Central Composite Design.
Note:- N.D is Not Detected

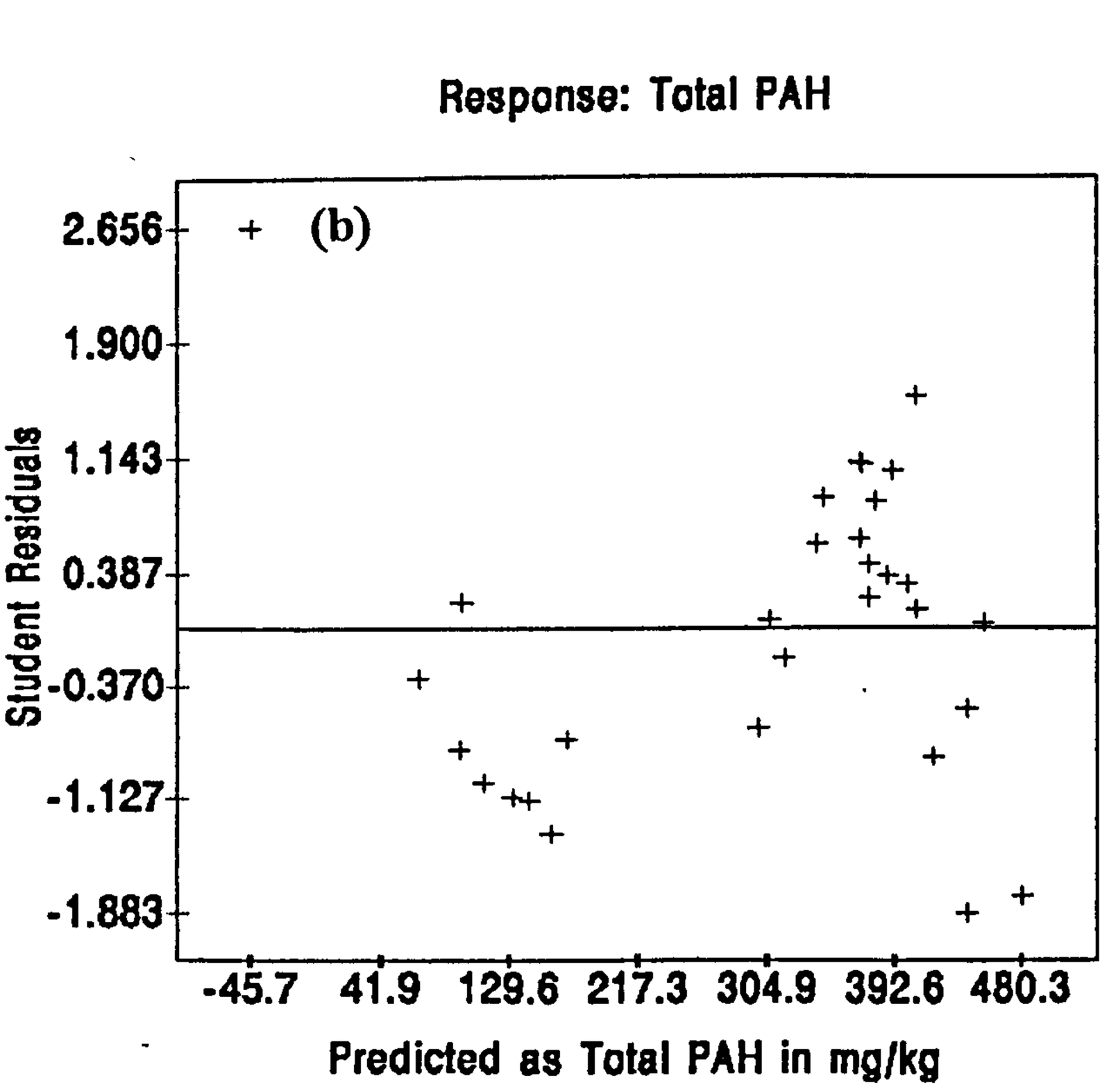
Compound	13	14	15	16	17	18	19	20	21	22	23	24
Naphthalene	8.9	10.2	9.8	9.3	5.4	9.7	3.5	10.3	12.2	9.1	9.0	10.7
Acenaphthylene	1.4	3.0	3.2	2.8	1.1	2.9	0.9	3.1	3.2	2.8	2.4	3.4
Acenaphthene	6.4	15.4	14.9	13.6	6.3	14.1	4.6	14.2	16.9	13.6	11.8	16.0
Fluorene	4.3	13.9	13.1	11.9	5.7	12.5	4.0	12.7	14.5	12.1	10.3	15.6
Phenanthrene	10.0	81.7	77.6	71.1	29.9	74.5	23.5	76.4	87.6	69.4	59.9	86.2
Anthracene	6.1	26.4	23.5	24.5	10.7	28.0	9.1	33.5	34.5	29.6	21.1	30.2
Fluoranthene	20.5	78.9	75.5	69.3	23.9	73.2	21.4	73.2	82.0	65.8	56.1	87.4
Pyrene	13.7	57.8	57.0	51.7	15.1	54.5	14.9	54.3	58.0	49.6	40.3	66.4
Benz(a)anthracene	4.0	24.7	25.8	22.9	4.1	23.6	5.8	24.1	24.6	21.3	16.2	29.7
Chrysene	4.9	26.5	27.2	24.5	4.3	26.1	6.2	26.8	26.9	23.3	17.9	32.0
Benzo(b)fluoranthene	2.6	14.8	19.3	15.5	1.8	11.6	1.9	14.8	8.4	12.5	6.4	19.6
Benzo(k)fluoranthene	4.1	16.4	15.8	15.6	1.0	18.0	3.3	15.8	14.5	16.2	10.9	12.8
Benzo(a)pyrene	4.1	19.6	22.6	20.3	0.9	19.4	2.4	19.8	17.8	17.8	8.8	25.6
Indeno(1,2,3-cd)pyrene	1.6	10.9	13.3	11.9	0.1	10.3	0.8	10.6	8.6	9.0	2.8	14.1
Dibenz(a,h)anthracene	0.2	1.5	2.3	1.8	N.D	1.5	0.2	1.4	1.3	1.3	0.6	1.9
Benzo(ghi)perylene	1.2	8.0	10.0	9.1	0.2	7.9	0.5	8.1	5.9	6.4	1.6	10.7
Total	94.1	409.7	410.9	375.8	110.5	387.8	103.0	399.1	416.9	359.8	276.1	462.3

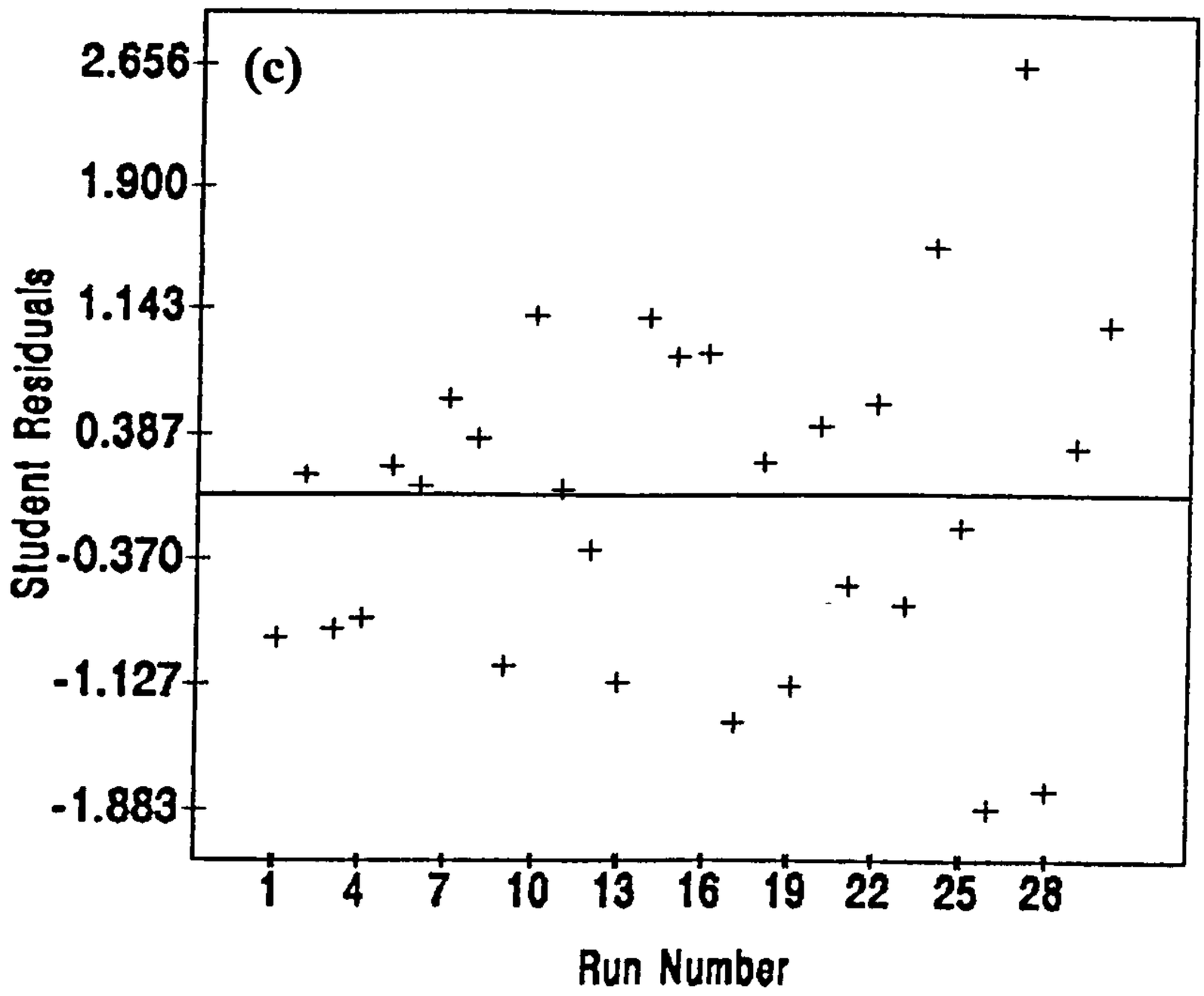
Table A5.12 Continued.

Compound	25	26	27	28	29	30
Naphthalene	7.5	9.4	2.5	10.3	9.8	16.1
Acenaphthylene	2.3	3.0	0.3	3.1	3.2	3.7
Acenaphthene	11.2	12.4	1.6	14.6	14.4	18.6
Fluorene	10.7	11.7	0.5	14.0	14.2	16.2
Phenanthrene	61.5	66.8	7.3	77.8	78.5	98.0
Anthracene	19.3	23.5	7.1	26.8	28.1	34.5
Fluoranthene	58.4	63.7	8.9	75.3	75.8	91.4
Pyrene	42.5	47.6	6.9	56.5	55.9	61.6
Benz(a)anthracene	19.6	21.2	2.7	25.5	24.6	22.5
Chrysene	21.1	22.9	3.0	26.8	26.5	24.4
Benzo(b)fluoranthene	15.2	12.0	1.2	19.8	18.7	14.2
Benzo(k)fluoranthene	8.5	15.3	1.5	18.1	16.9	10.1
Benzo(a)pyrene	16.2	17.3	2.2	22.6	21.7	9.4
Indeno(1,2,3-cd)pyrene	9.1	10.1	0.7	13.9	13.0	4.7
Dibenz(a,h)anthracene	1.6	1.9	0.1	2.4	2.4	0.7
Benzo(ghi)perylene	6.8	7.7	0.4	11.2	10.2	3.3
Total	311.5	346.5	46.8	418.7	413.9	429.4

Table A5.12 Continued.
(Concentration in mg kg^{-1})


Response: Total PAH
(a)
99
95
90
80
70
50
30
20
10
5
1
Normal % Probability
-1.883 -1.127 -0.370 0.387 1.143 1.900 2.656
Studentized Residual


Response: Total PAH
(b)
2.656
1.900
1.143
0.387
-0.370
-1.127
-1.883
Student Residuals
-45.7 41.9 129.6 217.3 304.9 392.6 480.3
Predicted as Total PAH in mg/kg



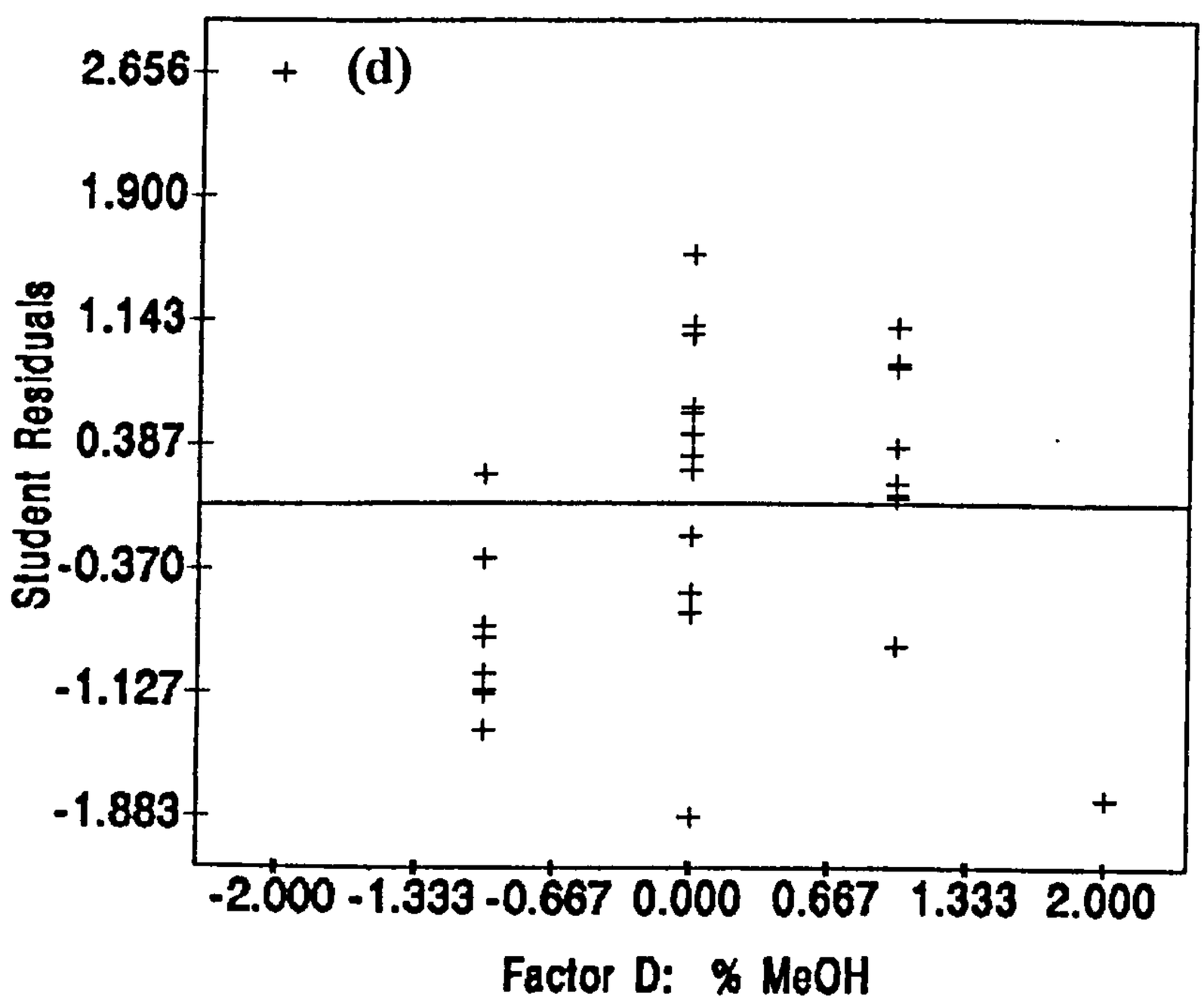


Figure A5.1 (a) Normal Probability *Versus* Studentized Residuals; (b) Studentized Residuals *Versus* Predicted Response; (c) Studentized Residuals *Versus* Run Number; (d) Studentized Residuals *Versus* % Methanol (coded).

Compound	1	2	3	4	5	6	7	Average	% RSD
Naphthalene	10.8	11.0	10.8	9.9	9.8	11.3	10.2	10.5	5.5
Acenaphthylene	3.5	3.5	3.7	3.8	3.5	4.2	3.3	3.6	8.1
Acenaphthene	15.4	17.1	17.9	16.1	16.2	17.2	16.2	16.6	5.1
Fluorene	12.5	14.5	15.7	14.3	14.0	15.7	13.3	14.3	8.2
Phenanthrene	83.9	91.4	96.3	83.8	86.3	91.2	83.8	88.1	5.6
Anthracene	30.5	35.4	35.8	30.8	31.0	35.1	30.2	32.7	7.9
Fluoranthene	78.7	84.5	87.5	75.6	81.2	75.3	77.9	80.1	5.7
Pyrene	58.6	62.8	64.5	59.6	60.7	61.9	63.6	61.7	3.5
Benz(a)anthracene	27.3	28.5	29.3	27.7	27.7	28.1	26.7	27.9	3.0
Chrysene	28.3	29.1	29.8	28.9	28.6	29.4	27.7	28.8	2.4
Benzo(b)fluoranthene	27.4	18.0	13.6	18.4	18.9	20.3	22.6	19.9	2.2
Benzo(k)fluoranthene	19.6	12.9	17.1	13.2	13.6	14.5	16.2	15.3	16.1
Benzo(a)pyrene	24.4	26.3	25.7	25.2	25.7	24.6	23.7	25.1	3.6
Indeno(1,2,3-cd)pyrene	16.6	18.4	17.7	17.5	18.6	16.9	16.2	17.4	5.2
Dibenz(a,h)anthracene	1.8	2.0	2.7	3.9	3.1	2.9	1.8	2.6	30.1
Benzo(ghi)perylene	12.6	13.9	13.6	13.3	14.3	13.1	12.5	13.3	5.0
Total	452.0	469.4	481.9	441.9	453.2	461.8	446.1	458.0	3.1

Table A5.13 Repeatability Study using the Central Composite Design Optimum Conditions.
(concentration in mg kg^{-1})

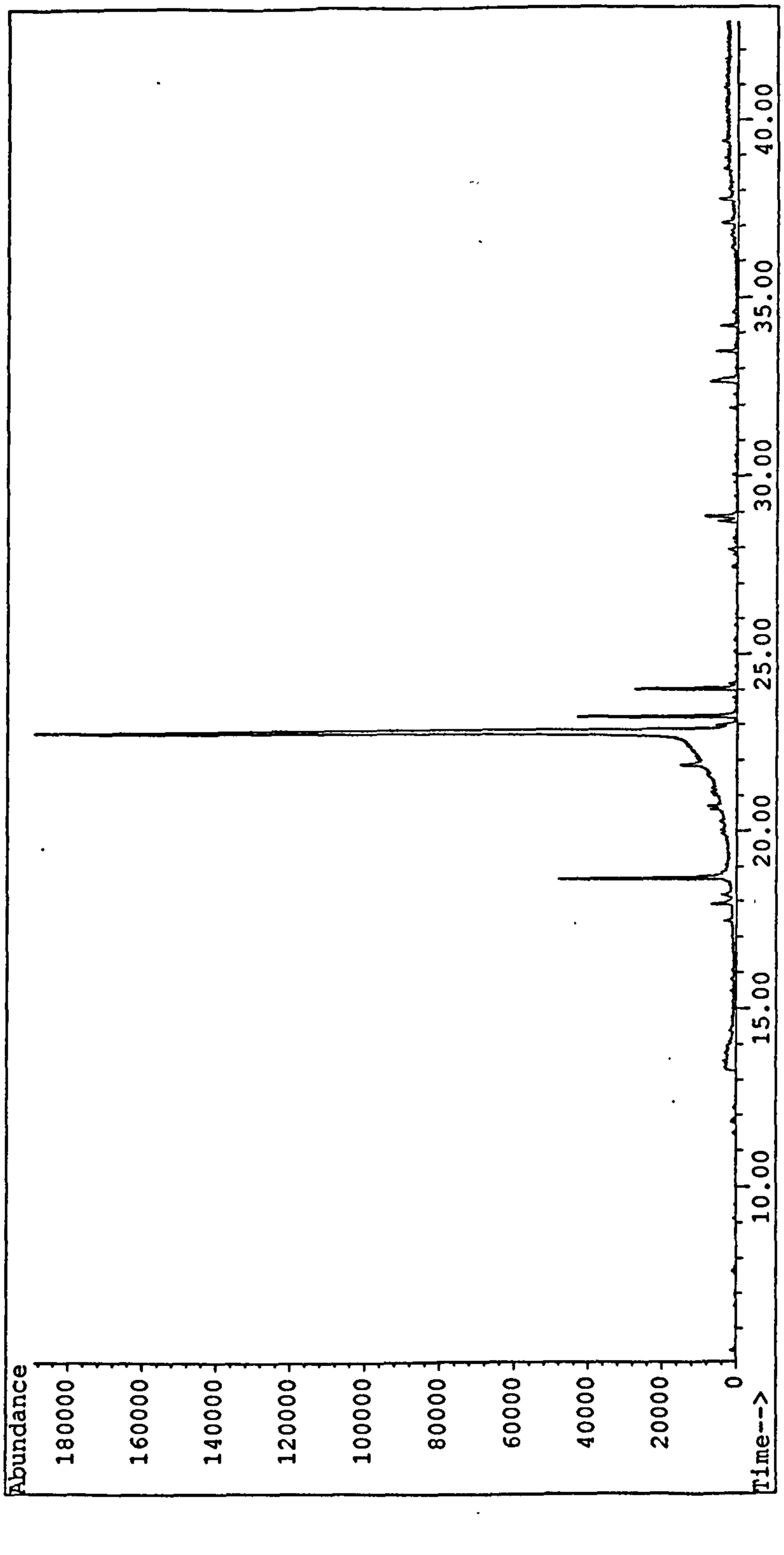


Figure A5.2 A Typical Chromatogram Obtained During SFE of the LGC CONTEST Soil.

Compound	1	2	3	4	Average	% RSD
Naphthalene	3.6	1.0	2.7	1.0	2.1	62.4
Acenaphthylene	0.6	0.7	0.5	0.6	0.6	13.6
Acenaphthene	0.3	0.9	0.7	0.6	0.6	40.0
Fluorene	0.2	0.3	0.2	0.2	0.2	22.2
Phenanthrene	74.6	70.0	45.4	64.5	63.6	20.2
Anthracene	70.3	69.9	53.8	75.2	67.3	13.8
Fluoranthene	49.0	49.3	39.4	53.1	47.7	12.2
Pyrene	31.8	31.3	26.0	34.6	30.9	11.6
Benz(a)anthracene	8.8	7.8	6.1	8.7	7.9	15.9
Chrysene	13.9	14.4	12.0	15.0	13.8	9.4
Benzo(b)fluoranthene	14.5	6.4	4.7	9.1	8.7	49.4
Benzo(k)fluoranthene	10.4	12.6	10.0	11.4	11.1	10.5
Benzo(a)pyrene	2.1	1.7	1.3	8.4	3.4	99.7
Indeno(1,2,3-cd)pyrene	9.1	20.1	14.6	20.1	16.0	33.0
Dibenz(a,h)anthracene	1.7	1.0	1.1	0.9	1.2	30.6
Benzo(ghi)perylene	6.6	6.3	4.2	6.2	5.8	18.8
Total	297.4	293.7	222.7	309.6	280.9	14.0

Table A5.14 Repeatability Study (CONTEST Soil Sample) using the Central Composite Design.
(concentration in mg kg^{-1})

Appendix 6

Sample	Depth (m)	Organic❶ %	Quartz❷ %	'Clay'❸ %	Al❹ %	Fe❹ %	S.G.*❺	CEC❻ (meq/100g)
L1	0.0-0.7	2.1	50	4.34	6.33	1.41	2.70	7.29
L2	0.7-1.0	2.2	62	3.34	6.81	1.85	2.70	3.89
L3	1.0-1.5	1.7	58	1.67	6.11	0.92	2.86	3.81
L4	1.5-1.8	0.8	42	N/A	3.17	1.74	2.83	5.40
L5	1.8-2.0	0.2	65	1.17	1.37	0.61	2.66	1.56
L6	2.0-2.25	0.4	62	N/A	1.79	0.61	2.66	2.18
L7	2.25-2.6	35.0	52	1.67	1.67	0.66	2.00	199.88
L8	2.6-3.1	15.0	34	2.51	5.98	2.20	2.00	24.47
L9	3.1-3.4	8.0	34	11.69	3.17	1.03	1.78	15.91
L10	3.4-3.6	4.0	32	5.01	2.74	1.76	2.80	13.44
L11	3.6-3.8	2.0	40	11.69	2.94	1.71	2.72	12.78
L12	3.8-3.9	1.0	32	23.38	3.67	1.05	2.75	9.78

Table A6.1 Soil Characterization.[342]
* Where S.G. is specific gravity.

❶ Determined by Thermogravimetry using 5 mg of an air-dried sample heated at 20 °C min^{-1}

❷ Determined by Differential Thermal Analysis with quartz reference

❸ Determined by sedimentation analysis of air dried soils

❹ Determined using direct current plasma - atomic emission spectroscopy after hydrofluoric acid microwave digestion

❺ Determined using a 10 ml specific gravity bottle

❻ Determined with barium as the indicator ion

Repeat Number	Lindane	Aldrin	Dieldrin	Heptachlor	Isodrin
1	92.4	85.9	85.4	91.8	83.4
2	89.1	86.8	86.2	92.6	84.2
3	92.8	90.2	92.4	97.3	88.8
4	104.7	93.9	102.9	93.0	96.4
5	93.3	81.3	85.1	102.1	96.4
Average	94.5	87.6	90.4	95.4	89.8
% RSD	6.3	5.4	8.4	4.5	7.0

Table A6.2 Percentage Recoveries of Organochlorine Pesticides from Celite.

Repeat Number	Diazinon	Malathion	Chlorfenvinphos
1	94.2	85.7	97.5
2	94.4	84.7	83.7
3	96.5	87.8	94.4
4	97.2	89.6	99.5
5	106.8	90.1	91.8
Average	97.8	87.6	93.4
% RSD	5.3	2.7	6.6

Table A6.3 Percentage Recoveries of Organophosphorus Pesticides from Celite.

Repeat Number	Simazine	Propazine	Trietazine	Chlortoluron	Isoproturon	Diuron
1	80.7	82.2	84.7	78.9	81.6	79.2
2	86.6	76.8	89.7	84.6	81.5	80.6
3	77.3	81.8	97.7	77.4	77.8	75.8
4	82.9	82.8	94.0	83.2	81.5	77.8
5	80.4	78.1	92.2	78.1	77.7	71.7
Average	81.6	80.3	91.7	80.4	80.0	77.0
% RSD	4.2	3.4	5.3	4.0	2.6	4.5

Table A6.4 Percentage Recoveries of both s-Triazine and Urea Herbicides from Celite.

Ext. No.	% Org.	Soil No.	Lindane	Aldrin	Dieldrin	Heptachlor	Isodrin
1	0.2	L5	59.0	72.1	46.4	58.9	48.2
2	0.2	L5	45.0	59.3	42.5	44.2	41.3
3	0.2	L5	-	-	-	-	-
1	2.0	L11	45.3	54.8	27.8	50.7	32.7
2	2.0	L11	53.0	59.4	31.1	56.1	38.1
3	2.0	L11	51.9	62.2	33.5	57.5	38.6
1	15.0	L8	43.4	58.5	43.6	45.2	41.6
2	15.0	L8	60.5	81.5	60.3	67.8	56.4
3	15.0	L8	51.7	69.8	49.2	59.4	49.1
1	35.0	L7	29.0	40.9	29.9	34.7	28.8
2	35.0	L7	32.6	47.7	33.7	40.3	33.7
3	35.0	L7	29.0	39.9	28.9	34.0	28.6

Table A6.5 Percentage Recoveries of Organochlorine Pesticides from Characterized Soils.

Ext. No.	% Org.	Soil No.	Diazinon	Malathion	Chlorfenvinphos
1	0.2	L5	87.9	52.5	55.6
2	0.2	L5	79.6	50.6	53.1
3	0.2	L5	86.1	60.1	62.8
1	2.0	L11	95.4	117.2	121.9
2	2.0	L11	93.5	90.5	100.6
3	2.0	L11	91.8	76.0	90.7
1	15.0	L8	98.4	78.5	83.5
2	15.0	L8	97.7	78.0	79.5
3	15.0	L8	94.0	72.4	74.0
1	35.0	L7	44.3	36.8	43.4
2	35.0	L7	49.9	50.1	55.8
3	35.0	L7	36.6	41.9	44.0

Table A6.6 Percentage Recoveries of Organophosphorus Pesticides from Characterized Soils.

Ext. No.	% Org.	Soil No.	Simazine	Propazine	Trietazine	Chlortoluron	Isoproturon	Diuron
1	0.2	L5	57.5	56.8	65.8	55.4	54.1	53.6
2	0.2	L5	58.5	55.8	74.9	56.3	56.6	59.8
3	0.2	L5	67.0	74.6	68.2	70.2	66.9	61.4
1	2.0	L11	65.6	55.5	59.3	56.2	55.6	51.6
2	2.0	L11	56.4	45.7	53.8	53.3	49.4	46.3
3	2.0	L11	71.7	76.9	61.9	65.4	61.5	44.5
1	15.0	L8	86.6	84.8	83.9	68.5	95.4	93.7
2	15.0	L8	68.5	61.5	66.5	66.0	72.4	75.5
3	15.0	L8	66.0	69.3	67.5	-	67.9	63.8
1	35.0	L7	70.1	63.7	61.9	66.8	62.5	58.0
2	35.0	L7	-	-	-	-	-	-
3	35.0	L7	76.5	78.4	57.5	68.6	65.5	65.0

Table A6.7 Percentage Recoveries of both s-Triazine and Urea Herbicides from Characterized Soils.

Appendix 7

Name of Unit	Symbol	Equivalent in Pascals (Pa)
bar	bar	10^5
atmosphere	atm	101325
pounds per square inch	psi	6.89×10^3
kilogrammes per square meter	$kg\ m^{-2}$	9.79×10^4

Table A7.1 Pressure Conversion Table.*

* Conversion factors obtained from Schwarzenbach et al.[1]